PREVENTION OF CONGENITAL TOXOPLASMOSIS IN THE NETHERLANDS

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PREVENTION OF CONGENITAL TOXOPLASMOSIS IN THE NETHERLANDS

PREVENTIE VAN
CONGENITALE TOXOPLASMOSE
IN NEDERLAND

PROEFSCHRIFT

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aan Bob
Thomas & Anaïs

aan Mevrouw Prien

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ABBREVIATIONS

CBS	Central Bureau of Statistics
CHT	congenital hypothyroidism
CT	congenital toxoplasmosis
CTI	congenital toxoplasma infection
DIF	direct immunofluorescent
ELISA	enzyme-linked immunosorbent assay
EM	electron microscopy
GG&GD	Gemeentelijke Geneeskundige en Gezondheidsdienst Municipal Medical and Health Service
GHI	Geneeskundige Hoofdinspectie Medical Office of Health
GVO	gezondheidsvoorlichting en opvoeding health education
GP	general practitioner
GP/M/O	general practitioner, midwife or obstetrician
IIF	indirect immunofluorescent
IgA	immunoglobulin A
IgG	immunoglobulin G
IgM	immunoglobulin M
NIPG	Nederlands Instituut voor Preventieve Gezondheidszorg Netherlands Institute for Preventive Health Care
PAP	peroxidase anti-peroxidase
PCR	polymerase chain reaction
PKU	phenylketonuria
PKV	Provinciale Kruisvereniging Home Nursing Organization
RIVM	Rijksinstituut voor Volksgezondheid en Milieuhygiëne National Institute of Public Health and Environmental Protection
SF	Sabin-Feldman
TIP	toxoplasma infection prevention

1 GENERAL INTRODUCTION

1.1 INTRODUCTION

Toxoplasmosis is an infectious disease that frequently occurs in humans and animals all over the world, except on Antarctica. It is caused by *Toxoplasma gondii*, a protozoan. The parasite was described for the first time in 1908 by Nicole and Manceaux who identified it in a rodent from North Africa, the *Ctenodactylus gundi* (figure 1.1). It was not until 1923 that the parasite was recognized during autopsy in the eye of a human infant.[1] In 1939 Wolf described a fatal neurological disease attributable to *Toxoplasma gondii* in three neonates.[2] After introduction of the so-called dye test of Sabin and Feldman,[3] the epidemiological aspects of toxoplasmosis could be further investigated and the clinical spectrum in man established.

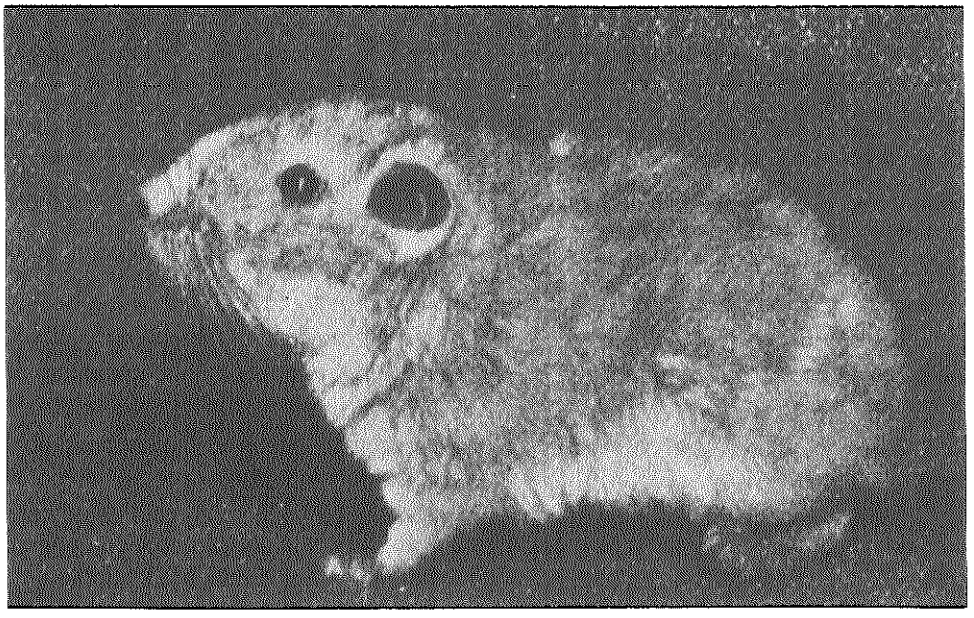

Figure 1.1 *Ctenodactylus gundi.* Brehms Tierleben. Leipzig/Wien, 1914.

Since then the parasite has been demonstrated in many animal species. A historical review on toxoplasmosis was published by Feldman in 1968.[4,5] In 1970 the life cycle was further elucidated when the special role of cats and other felines (Felidae) as host for *Toxoplasma gondii* was recognized: in these hosts *Toxoplasma gondii* can complete its cycle by sexual reproduction.[6-10] As a result of these parasitological studies, reviewed by Frenkel,[11] the nature of this zoonotic disease was better understood. Soon the importance of cats for the transmission of toxoplasma infections was assessed by means of epidemiological investigations.[12,13]
Both nationally and internationally the impact on the health of man, especially the unborn child, is being discussed. With respect to pregnancy some countries have a government-regulated system of measures to prevent or to trace and treat infection.
Within the scope of the present study on the occurrence and prevention of toxoplasma infections in the Netherlands, relevant data from literature will be reviewed in this chapter.

1.1.1 BIOLOGY OF *TOXOPLASMA GONDII*

Toxoplasma gondii, a coccidian, is an obligatory intracellular protozoan.[14] It may infect a broad range of warm-blooded hosts. Man and many animals, e.g. mice, rats, cattle, pigs, sheep, goats and poultry, are intermediate hosts in which Toxoplasma can multiply asexually.[15] The cat and other felines (Felidae) are the definite host for Toxoplasma, i.e. in these hosts the parasite can complete its reproductive cycle by sexual multiplication.[7] Toxoplasma isolated from different species are biologically and immunologically identical; only one variant of the parasite is known, *Toxoplasma gondii*, although the isolates may differ in virulence.
Asexual multiplication, also called the exo-enteral phase of the cycle, takes place in every type of cell in the intermediate hosts, except red blood cells. The tachyzoites, the proliferative form of *Toxoplasma gondii*, penetrate host cells and multiply intracellularly by endodyogeny: this is a process of multiplication in which two daughter cells originate within the mother organism which ultimately ruptures.[16] Subsequently the free young parasites enter neighbouring cells and/or are dispersed via the bloodstream or lymphatic system throughout the organism. After a while the offensive force of the parasites will be balanced by the growing defensive forces of the host (both cellular and humoral immunoresponse), which results in the

formation of cysts in the tissues. These cysts, which have a tough argyrophylic PAS-positive wall, normally lie quiescent in the tissues without causing any inflammatory reaction. Within the cysts are tens to hundreds of inactive slumbering forms of Toxoplasma, the so-called bradyzoites; thus the micro-organism persists. The presence of cysts in tissues characterizes a latent toxoplasma infection.
Reactivation of a latent infection may occur incidentally: if a cyst ruptures and parasites get out, immunity is challenged. Depending on local immunity the parasites are confined or invade contiguous cells, forming so-called satellite cysts; as a reaction to the antigens released, a hypersensitive reaction may develop with cell decay and tissue necrosis. Cyst rupture acts on immunity as a booster.[14]
Tachyzoites have an arch-like shape which is the basis for the name of the parasite: toxon (*τοξον*) is the greek word for arch; they measure 4 to 7 μ by 2 to 4 μ. Figure 1.2 shows them as fluorescent structures in a direct immunofluorescent (DIF) assay; in figure 1.3 they are visualized by electron microscopy (EM). Figure 1.4 shows a tissue cyst in homogenized brain material from an infected mouse.
Sexual multiplication takes place in epithelial cells in the small intestine of the definite host. It is also called the entero-epithelial phase of the life cycle. Tachyzoites invade the cells and multiply intracellularly by schizogony. Merozoites originate and, after disruption of the cell, invade other epithelial cells. Moreover gametocytes can develop from merozoites and subsequently produce microgametes and macrogametes by gametogony. Fusion of a microgamete and a macrogamete yields a zygote which subsequently becomes an oocyst. The Scottish parasitologists Hutchison and coworkers, who had studied the possibility of faecal transmission by cats since 1965, presented in 1970 conclusive evidence that *Toxoplasma gondii* is in fact a coccidian that undergoes typical schizogenic cycles in the intestinal epithelial cells of the cat.[6] Oocysts are excreted in large numbers with the faeces and thus enter the environment. One to five days after excretion the oocysts sporulate; two sporocysts, each of which contains four sporozoites, are formed during this sporogony; these sporulated oocysts are infectious and remain so for very long periods (up to two years), since they are highly resistant to various climatological and physical conditions. Oocysts, which measure 10-12μ, are shown in figure 1.5.
In table 1.1 some characteristics of the reproduction of *Toxoplasma gondii* in intermediate and definite hosts are listed.

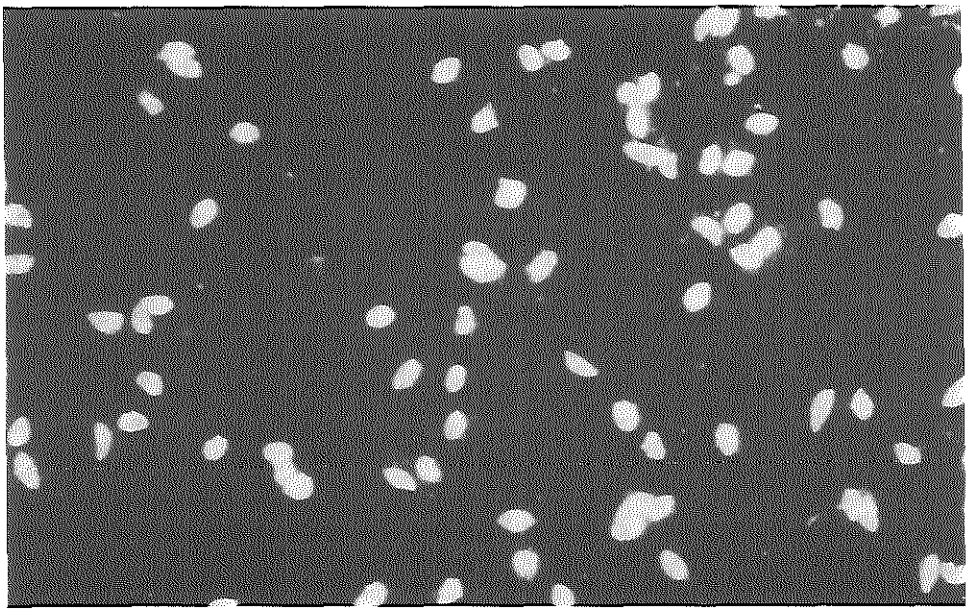

Figure 1.2 *Toxoplasma gondii* by direct immunofluorescent assay.

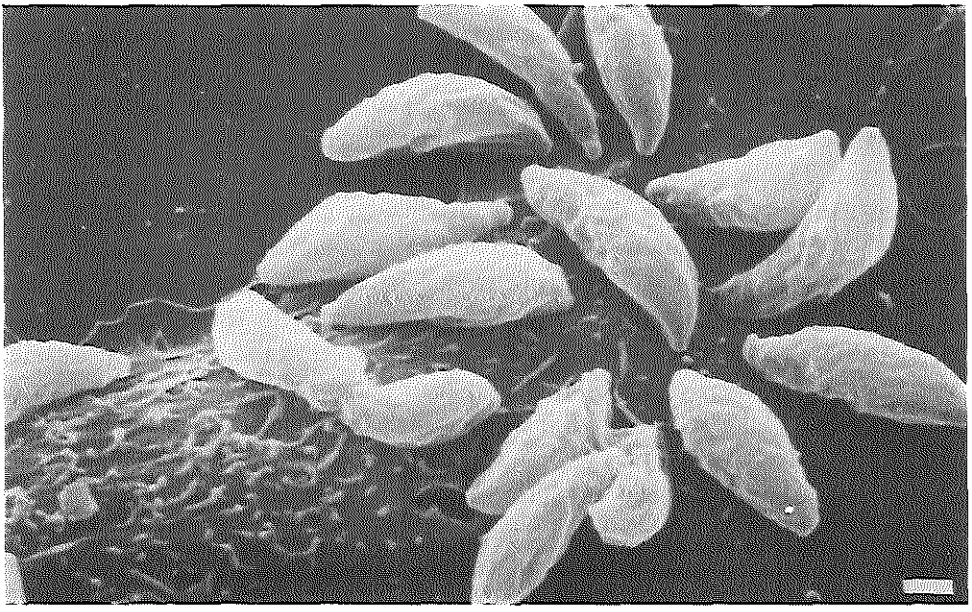

Figure 1.3 *Toxoplasma gondii* on the surface of a spread macrophage by electron microscopy; bar = 1μm (Drs. J.F. Teppema, Laboratory for Pathology, RIVM).

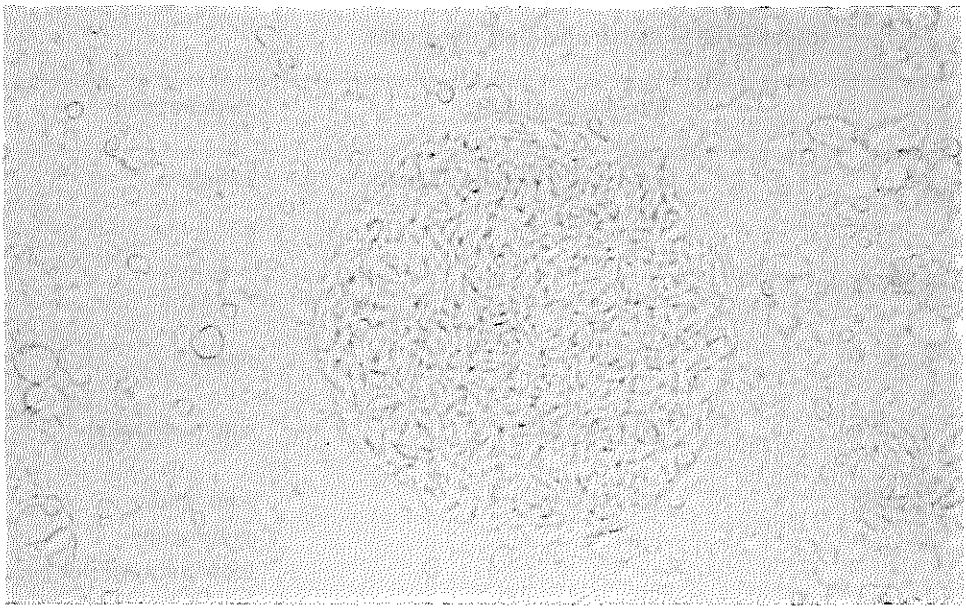

Figure 1.4 Tissue cysts with many bradyzoites of *Toxoplasma gondii* in homogenized brain material of an infected mouse (unstained).

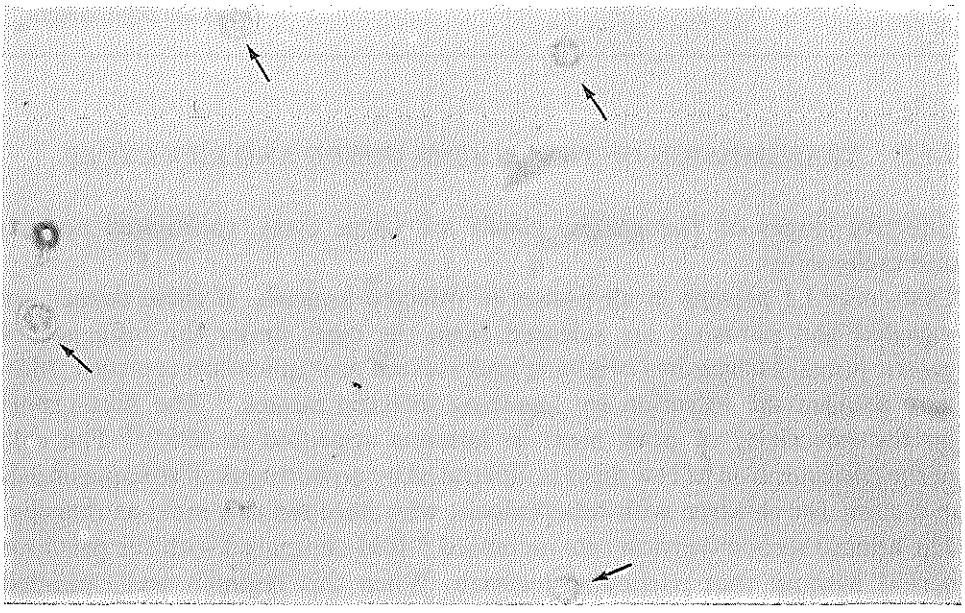

Figure 1.5 Oocysts in faecal material (unstained)

Table 1.1 Some characteristics of the reproduction of *Toxoplasma gondii* in intermediate hosts and definite hosts.

	Intermediate hosts	Definite hosts
host	man, warm-blooded animals incl. Felidae	Felidae
reproduction:		
type	asexual	sexual
location	extra-intestinal	entero-epithelial
process	endodyogeny	schizogony gametogony sporogony (outside host)
endstage	cysts full of bradyzoites	oocysts
infective stage	cysts in infected tissues	sporulated oocysts from the environment

1.1.2 TRANSMISSION OF *TOXOPLASMA GONDII* AND LIFE CYCLE

Infective stages of *Toxoplasma gondii* are tissue cysts, sporulated oocysts and tachyzoites.
Cysts may be present in all tissues of infected animals. If these tissues are ingested and parasites are released from the cysts under the influence of gastric juices, tachyzoites originate and asexual multiplication can start. Man may acquire the infection by ingesting infected tissues.[17] The first report on the infectivity of pork meat in humans appeared in 1955.[18] The role of the consumption of raw meat is illustrated by an outbreak of toxoplasmosis in a clinic for children with tuberculosis, who were fed raw meat as therapy. The annual seroconversion rate increased 5-fold compared with the previous period without that particular regimen. When this regimen was extended to include fortnightly raw sheep meat the rate doubled.[19]

Also, an outbreak in the USA caused by 'hamburger' consumption was reported.[20] Cysts, however, cannot resist high temperatures; therefore thorough heating of the meat before consumption destroys the infective cysts. But they remain contagious for a long time in cooled meat. Freezing to –20°C for more than 48 hours, however, is sufficient to kill the cysts.[21] Gamma irradiation (Caesium-137 or Cobalt-60 at 50,000 rads) is effective as well.[22]

Tissue cysts in prey and raw meat as well as oocysts in the environment that have sporulated can be taken up orally by cats; sexual multiplication will then begin in the small intestine, resulting in faecal excretion of enormous numbers of oocysts. Oocysts are killed by exposure to a temperature of 55°C for 30 minutes. Humidity and low temperatures seem to enhance survival. Since cats bury their faeces, cysts may survive for prolonged periods in humid soil. Furthermore they are resistant to acid, alkali and the usual detergents.[10,23] Oocysts in the environment are an important source of infection of man and animals. A route of transmission, that probably is extremely rare, is oocyst-contaminated drinking water. One such epidemic occurred among soldiers in the Panama jungle.[24]

In addition to these common routes, an acquired toxoplasma infection can result from parenteral administration of parasites, i.e. inoculation of tachyzoites accidentally in the laboratory or iatrogenic transmission by means of blood transfusions or transplantation of organs that contain cysts.[25,26] In the latter case infection will be enhanced by concomitant immunosuppressive therapy.

Tachyzoites may also be transmitted transplacentally to the foetus; this vertical transmission leads to congenital infection. Horizontal transmission in man has not been reported.

In figure 1.6 the life cycle of *Toxoplasma gondii* is depicted.

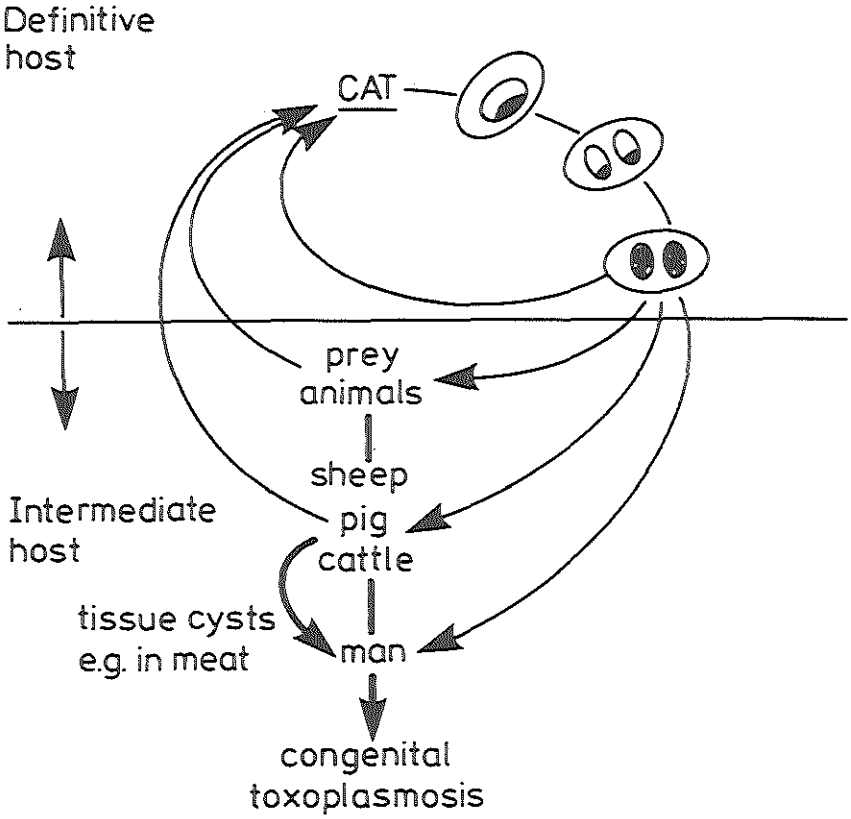

Figure 1.6 Life cycle of *Toxoplasma gondii*

1.2 PREVALENCE OF TOXOPLASMA INFECTIONS IN ANIMALS

Toxoplasma gondii has been found in many animals, but the prevalence varies from species to species. Very often infection is asymptomatic but if clinical illness develops, the symptoms are almost identical in different species. There may be lymphadenopathy and hepatosplenomegaly. The most important manifestations are encephalitis, pneumonia and myositis; enteritis may also develop. Infection of pregnant animals often causes abortion, premature birth and stillbirth. Moreover congenital infection of the offspring is observed in many animals, such as sheep, rabbits and mice.

An extensive report on the clinical and pathological aspects of toxoplasma infection in animals is given by Siim et al.[27]

Since infection is so often asymptomatic, it is not possible to derive information about the occurrence from clinical diagnosis. Incidence data

can only be obtained by prospective studies involving repeated serological testing. Data on prevalence are available from serological and parasitological studies of different species.[17] The first report was published in 1957.[28]
Because of the importance of the transmission of toxoplasma infection from animal to man by meat consumption, the prevalence in some relevant species in the Netherlands will be discussed in this section.

1.2.1 LIVESTOCK

CATTLE

In 1958 a seroprevalence of 20% was reported; in the late sixties and in the late seventies this figure had not changed.[29,30,31] Beef is probably not as important as a source of infection as was originally assumed since parasites can seldom be isolated from seropositive animals whereas antibodies can be measured only at a low level and for a limited period in seropositive cattle.[32,33] Even after experimental infection isolation of parasites was possible only for a short period; none could be isolated from the organs of healthy animals.[23] Whether infection of cattle results in persistent chronic infection and whether it provides the animal with resistance against subsequent exogenous infection still have to be investigated. Transmission to human beings via milk seems to be conceivable, but it is likely that pasteurization of milk would destroy all forms of Toxoplasma.[34,35] The WHO Working Groups "Husbandry, Household and Environment" and "Food Hygiene", that considered the public health aspects of toxoplasmosis (Meeting, 23-24 October 1989, Bilthoven, the Netherlands), stated that beef – as such – does not constitute a public health hazard. An exception might occur if the beef were to be mixed with other infected meat.

PIGS

Pork is considered to be an important source of toxoplasma infection.
Serological data on the prevalence of toxoplasma infection in pigs vary considerably. In the Netherlands a striking decrease in the seroprevalence for pigs was observed.In 1958 48% was found to be seropositive,[29] in 1963 62%[36] and in 1969 54% of bacon pigs and 86% of sows;[30,36] in 1982, however, no antibodies were demonstrated in 200 bacon pigs and 11% of 36 sows was seropositive.[31] Such shifts have also been encountered in other European countries.[37-40] Studies from different countries were reviewed by Dubey.[35] The shift reflects the effect of changes in livestock breeding: animals remain

inside buildings that are kept free of cats, mice and rats. Animals allowed outside are much more likely to catch an infection than those who are raised inside (Van Knapen, personal communication). Thus industrialized farming seems to aid the prevention of toxoplasma infection.

SHEEP

The prevalence of infection in sheep can be estimated from serological data. Data from 1982, namely 30% seropositivity, contrast with earlier data: 65% in 1958, 89% in 1963 and 92% in 1970.[29-31,36,41]
In the past samples were taken from older breeding sheep; in recent studies young animals, destined for consumption, were sampled. This explains the reported difference, as could be confirmed in a 3-year follow-up study of a sheep flock that finally yielded a prevalence of 100% (Van Knapen, personal communication).

HORSES

In 1958 a prevalence of 7% was reported for Dutch horses,[29] and no changes were seen in 1979.[31] The impact of consumption of horse meat remains to be investigated.

POULTRY

The first prevalence figures for fowl are from 1979: 30% seropositivity for free-range chickens, 0% for broilers and laying hens.[31] Eggs as a source of infection have been excluded.[42,43]

Meat products (e.g. different types of sausages) generally are considered to be less hazardous than fresh meat because of the preservation techniques, such as salting, low pH, drying, etc.
The question arises whether meat of infected livestock, as assessed at the slaughterhouse, should be prohibited for consumption.[44] Freezing may destroy cysts in infected meat to a great extent, but this can never guarantee that there are no more viable cysts.[45] Gamma radiation (Caesium-137 or Cobalt-60) at 50,000 rads can render tissue cysts in meat nonviable;[22] but irradiation has not been generally accepted by the public in the Netherlands up to now. Another possibility might be to bring certified toxoplasma-free meat onto the market for consumption by certain risk groups (e.g. immuno-suppressed patients and pregnant women).

1.2.2 CATS

Cats are a cardinal factor in the epidemiology of toxoplasma infections. If there were no cats, there would be no toxoplasmosis. However there are two rather peculiar reports concerning cats as the source of infection: one outbreak among several adults who frequently visited a riding stable; they presented with symptoms of acquired toxoplasmosis; there was no other common exposure than the stable where several cats were living.
Nevertheless oocysts could not be found in the soil at the time of the investigation and oocysts were assumed to be the source of infection merely on epidemiological grounds.[46] The other outbreak concerned young children of an extended family who also exhibited a high rate of clinical manifestation of acquired toxoplasmosis; all played in the same yard as a seropositive cat who had had a litter of six kittens, several with a non-specific illness and one that died. There was a significant association between illness and geophagia ("pica"). Again oocysts could not be found in the yard at the time of the investigation.[47] Epidemiological data correlating toxoplasma antibodies with exposure to cats are not conclusive.[48] This is explained by the fact that their role in the infection of man does not stem from direct contact.[49,50] Because of their licking, faecal matter is rarely found on cat fur. The probability of transmission to human beings via touching or caring for a cat is minimal.[51] Cats produce oocysts only for a short period (2-3 weeks) after primary infection. While 64% of a population of cats was seropositive, only 0.4% excreted oocysts.[52] The role of the cat in the infection of other hosts can be explained by contamination of the environment with oocysts which – after sporulation – remain infective for very long periods (up to two years).[53,54] Vaccination of cats in order to block the transmission of infectious stages of *Toxoplasma gondii* to man is not yet feasible. Intensified research in order to develop a vaccine for cats has been proposed;[55,56] but it must be realized that if a vaccine were to be available, only part of the cat population -only the less risky group of domestic cats- would undergo vaccination; the stray cats would not. Therefore the efficacy of the vaccination of cats in order to prevent congenital toxoplasmosis in man is doubtful.

1.2.3 IMPLICATIONS FOR TRANSMISSION

From the foregoing facts it is apparent that the rate of infection of animals is influenced by several factors, such as the way in which they are kept and the age of the animals.

Cats that are kept inside the house and are fed canned food instead of raw meat will not become infected very easily. Stray cats, however, all become infected soon after weaning and will contaminate the environment.[10,57,58] The increasing trend in the Netherlands today is to put livestock back out to pasture, which could be disadvantageous for toxoplasma infection prevention.
Moreover the risk of meat depends on the food habits of the consumer. The dietary habits of man are changing too: consumption of raw or insufficiently heated meat is becoming increasingly popular in the Netherlands; especially raw pork and mutton are unsafe; but mutton is consumed much less than lamb, which is less risky.
So there is a dynamic situation; no overall figure for the risk of meat consumption can be given. A definite statement about whether oocysts or tissue cysts constitute the most important source of infection also cannot be made.[7,12,59-62] This is highly dependent on the climatological, sociological/ behavioural and veterinary circumstances. In Western Europe infection is thought to be caused predominantly by ingestion of tissue cysts in meat.[7]

1.3 TOXOPLASMA INFECTION AND TOXOPLASMOSIS

A toxoplasma infection is a rather everyday event. As in animals, however, it is seldom clinically recognized in man and thus its importance is often underestimated. Therefore two contrasting terms are proposed: *'toxoplasma infection'* for the situation in which a person becomes infected but has no complaints or overt clinical signs, as distinct from *'toxoplasmosis'* for the situation in which the infection leads to clinical manifestations.
Again, the frequency of infection is measured by serological testing of populations, mainly in seroprevalence studies that yield indirect estimates of the incidence. Medical interest in *Toxoplasma gondii* has grown in the last decades due to the appearance of certain types of manifestation (for example, in immunosuppressed patients) on the one hand and prospective studies on the course of congenital infections on the other. The following section deals in succession with acquired toxoplasma infections, latent toxoplasma infections and congenital toxoplasma infections.

1.3.1 ACQUIRED TOXOPLASMA INFECTION

When *Toxoplasma gondii* invades a non-immune host, it leads to a generalized infection, a so-called 'primary' infection. During the active phase of the infection the host develops immunity; under the influence of cellular and humoral responses the parasites are forced into cysts and infection becomes latent.
In the course of a primary infection immune cells are activated; specific antibodies are thought to persist throughout life or at least for a considerable period of time.[14,63] The resulting immunity protects the host against exogenous and endogenous reinfection; escaped parasites are quickly localized and destroyed, so that a generalized infection will not develop.
Repeated inoculation only boosts immunity. Although it cannot be stated that the mere presence of specific antibodies protects the host against new infections, they are interpreted as a sign of a previous infection which indicates that both cellular and humoral immunity exist.

1.3.1.1 CLINICAL PICTURE

A primary toxoplasma infection is generally asymptomatic;[64] some aspecific complaints of an influenza-like nature may occur, such as malaise, fatigue, headache and anorexia. Lymphadenopathy is the most common clinical sign; often it is suboccipital and cervical but it may be more generalized.[65] Hepatomegaly and splenomegaly are observed and the picture may resemble infectious mononucleosis (Pfeiffer's disease). Moreover any organ may be affected; pneumonia, hepatitis, polymyositis, myocarditis or meningo-encephalitis may be seen but are very rare. Infection during pregnancy can be responsible for spontaneous abortion or for immature or premature delivery. There has been much debate about whether toxoplasmosis is responsible for repeated abortions; as yet none of the studies has led to a definite conclusion.[34,65] Repeated vertical transmission through successive generations has been found in animal models[66,67] but was never confirmed in man.

1.3.1.2 PROGNOSIS

Toxoplasma infections are self-limiting and become latent within several

weeks, resulting in persistent immunity. In a minority of cases, infection has a protracted course with persistent complaints, particularly fatigue and malaise. This is characteristic of chronic toxoplasmosis: the infection seems to be active from time to time and immunity does not seem to be able to convert it into latency. Therapeutic support is often ineffective. But generally, toxoplasma infections have a good prognosis and collapse of the balance between parasite and host with activation of a latent infection seldom has consequences because immunity suppresses it efficiently.

1.3.1.3 RISK

The risk of acquiring a toxoplasma infection depends on whether one has ever been infected before and one's behaviour, i.e. exposure to contaminated sources. Prospective studies to investigate incidence are scarce. Generally the risk of infection in a population is derived from age-specific seroprevalence figures.[68] The overall seroprevalence in a population gives information about the frequency of infection. But, epidemiologically of greater importance is the age-specific prevalence, especially the difference in prevalence between successive age groups which in turn yields age-specific risks of infection.[69] The increase in seroprevalence with age in such cross-sectional studies is evaluated as if it was derived from longitudinal observations. It is therefore interpreted as being the result of intercurrent infections during a particular period before the person moved to the successive age category. Not the seroprevalence but changes in seroprevalence reflect the number of infections to be expected.[70] Thus the risk of infection for the child-bearing age group may be the same in two populations with very different levels of seroprevalence.
Interpretation of the seroprevalence curves in this manner is based on the assumption that there are no cohort effects: no effects on seroprevalence that do not affect all cohorts to the same extent. However, if the individuals of a specific birth cohort were once exposed massively to a high force of infection[a] and those of a younger cohort were not, then the seroprevalence for the former will be relatively higher and the difference in seroprevalence with respect to the younger group will not provide the right information about the risk of infection during the interval between these two groups.

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[a] A parameter termed 'force of infection', employed by Muench in 1959 and defined as the instantaneous per capita rate at which susceptible individuals acquire infection.[69]

Van der Veen and Polak reported on Dutch seroprevalence data obtained from a study of 1661 persons in 1972-1976: seroprevalence rose by 40% in the interval between 20 and 40 years of age.[71] A sentinel study in 1980 of 720 subjects yielded comparable figures.[72] Therefore toxoplasma infections are fairly common in our country, especially among young adults.

1.3.2 LATENT TOXOPLASMA INFECTION

A latent toxoplasma infection is the more or less stabile end phase of an acquired toxoplasma infection characterized by persistent specific antibodies and the presence of tissue cysts with slumbering parasites (bradyzoites). Nevertheless the equilibrium between host and parasites might occasionally be disturbed. As a rule the situation normalizes as soon as the liberated parasites have boosted immunity. Such a short exacerbation seldom causes complaints. If, however, there is a severe impairment of immunity, reactivation of the infection will have very serious consequences. Here the parasite reveals itself as an opportunist. Immunity may be impaired iatrogenically: immunosuppressive therapy will reduce protection against both exogenously acquired infections[73] and endogenously reactivated latent infections.[25] In addition, in the case of transplantation, a non-immune recipient of an organ that contains *Toxoplasma gondii* may acquire infection.[25] Moreover the disease has frequently been observed during the last decade in AIDS patients.[74-77] The clinical picture is a fulminant generalized infection, dominated by neurological symptoms due to panencephalitis with extensive necrosis of the brain. If recognized in time it can be cured by antitoxoplasmotic therapy.

1.3.3 CONGENITAL TOXOPLASMA INFECTION

If a primary toxoplasma infection is acquired by a pregnant woman, parasites may be transmitted transplacentally to the foetus and cause congenital infection. It is generally assumed that there is no risk for women who were infected earlier in life.[78-81] Transmission from a latent maternal toxoplasma infection is very rare and mostly due to a concomitant immunosuppressive disease.[82] In the event of an acquired maternal infection parasites are not always transmitted; the rate is dependent on gestation, infections early in pregnancy being transmitted less frequently than infections late in pregnancy. The consequences of foetal infection are just the opposite: early infection in the vulnerable phase of the development of brain and organs is far more

serious than late infection. Congenital toxoplasmosis may be overt at birth, but very frequently there are no symptoms at that time. As proposed before, it is better to use the term congenital toxoplasma infection for the latter condition. Figure 1.7 gives a flow chart for toxoplasma infection during pregnancy.

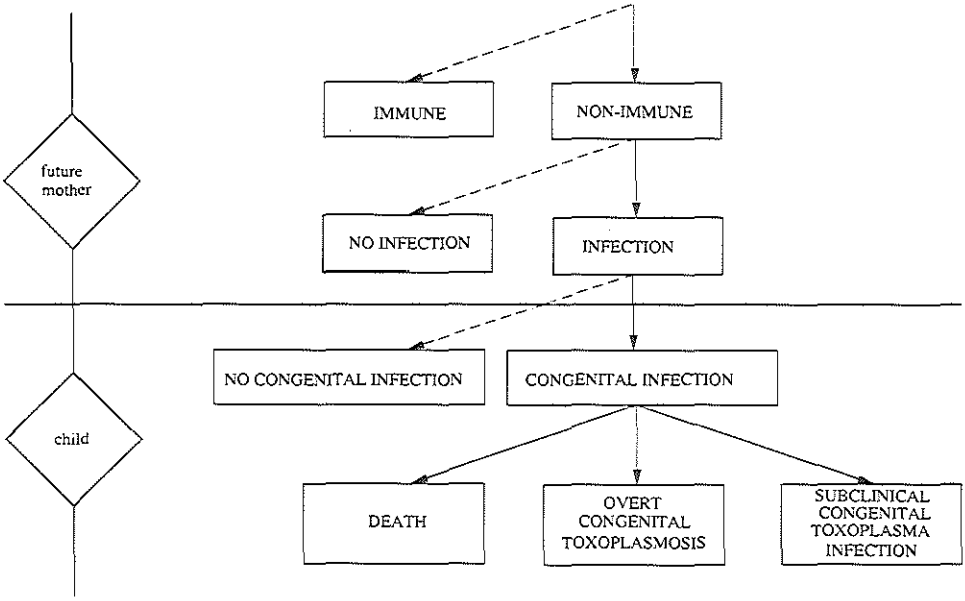


Figure 1.7 Flow chart for toxoplasma infection during pregnancy

1.3.3.1 CLINICAL PICTURE

Classically, congenital toxoplasmosis is characterized by the triad: hydrocephaly, intracerebral calcifications and chorioretinitis. These symptoms result from tissue decay in the vulnerable nervous system and eyes. But, *Toxoplasma gondii* is not organotropic; infection with the parasite may thus cause a very wide range of symptoms, often with so little specificity that the toxoplasmotic origin was not even considered in earlier times. Prospective studies have revealed that the above-mentioned triad is only the tip of the iceberg representing all possible symptoms of congenital toxoplasma infection; moreover it was found that clinical manifestation can be delayed for months or even years.[83-87] Which symptoms are to be expected depends greatly on the moment during pregnancy that infection took place.[79]
Early foetal infection commonly leads to more serious defects. At birth

– when the infection has already become latent – the defects present as the remaining signs of inflammation (intracranial calcifications, hydrocephaly due to obstruction of the circulation of cerebrospinal fluid caused by scar tissue, convulsions) and developmental disorders (microcephaly, microphthalmia, blindness).
Late foetal infection may still be active at birth, causing an acute fulminant inflammatory process with fever, unstable temperature, exanthema, thrombocytopaenia, anaemia, icterus, hepatosplenomegaly and abnormal cerebrospinal fluid. Abnormalities of cerebrospinal fluid without neurological findings were encountered in a large number of cases by Alford,[85] and its importance for recognition of congenital disease has been stressed by others,[88] but lumbar puncture is seldom performed when the disease has not yet been diagnosed.
However, at birth there usually is no sign of the congenital infection at all. In addition to serious involvement of the central nervous system, minor cerebral damage due to congenital toxoplasmosis might be responsible for even 20% of all mentally retarded children. This hypothesis is based on the relatively high prevalence of toxoplasma infections among the mentally retarded. However, the study of Thalhammer involved non-homogeneous study groups and lacked appropriate controls.[89,90] Prospective studies from the United States also revealed later neurological sequelae with retardation and intellectual defects in children initially asymptomatic.[84,85,87] In a Dutch study of Koppe et al, the 12 infected children did not differ in school performance from 117 controls.[86] None was mentally retarded. Another European study also could not demonstrate differences in mental development.[91] This question may be clarified by long-term continuation of prospective studies and by investigations using animal models; in a mouse model of congenital toxoplasmosis used to study the pathogenesis of ocular toxoplasmosis, subtle behavioural deficits have been found.[92]
Deafness has been reported[83] but seems to be confined to severely diseased children with central nervous involvement. Sever points out the statistical relation between hearing loss in children and seroconversion or high antibody titres in the mother during pregnancy.[93] The validity of this and comparable studies is doubtful because of the (lack of) controls. There are no satisfactory data that prove that congenital toxoplasmosis is a significant cause of deafness.[94]

1.3.3.2 PROGNOSIS: OCULAR TOXOPLASMOSIS

The prognosis of a congenital infection is poor. In addition to the ocular signs of congenital toxoplasmosis already mentioned, ocular symptoms occurring later in life are to be feared, even in an initially asymptomatic person.[95] In contrast to that in postnatally acquired infection, immunity in congenital infection is apparently not capable of combating later exacerbation of the latent infection efficiently. Every relapse causes further damage, especially in immunologically isolated tissues, e.g. the central nervous system and the eyes. Loss of visual acuity is the most common symptom. Funduscopy reveals a characteristic focal necrotizing chorioretinitis. During the inflammatory phase, which is hypersensitive in nature, an exudate clouds the vitreous and fluffy, cotton-like patches are seen at funduscopy. Later these become sharply demarcated and (partly) pigmented to form a chorioretinal scar. New satellite lesions of chorioretinitis in the proximity of an old scar are typical and may lead to progressive visual impairment, depending on the location of the lesion.
Other less specific concomitant ocular signs have been reviewed by de Jong.[96]
The differential diagnosis should include other infectious agents such as cytomegalovirus. Determination of antibodies is of limited use, since a large proportion of the general population has antibodies, and antibodies due to congenital infection may have decreased to a low level.
It is recommended that undiluted sera be used to test for ocular toxoplasmosis.[97] Activity of ocular disease is not reflected by the antibody titre and reactivation of ocular lesions is not accompanied by changes in serum;[98] there is only an increased local production of antibodies in the eye.[99] If the typical clinical picture is obscured by, for example, an exudative reaction in the vitreous, the aqueous humour can be tested to assess intraocular antibody production.[100] It is generally accepted that toxoplasmic chorioretinitis is a sign of congenital infection, even if it appears in adult life.[81] Nevertheless reports on familial ocular toxoplasmosis ascribed to acquired infection in Brazil where the ingestion of raw pork contaminated with toxoplasmic cysts is common, must be mentioned.[101]
Koppe et al. performed a long-term follow-up study of 12 neonates who were known to have congenital toxoplasma infection.[86] In all cases primary infection of the mother was proven in a prospective screening programme that covered 1821 pregnant women. At birth 4 babies had a chorioretinitis scar while parasites were isolated from the placenta and cerebrospinal fluid of 1 child. In 7 other cases diagnosis was based on the persistence of specific

antibodies after the age of 2 years. After a 20-year follow-up 4 of the 5 cases diagnosed at birth had suffered recurrence of chorioretinitis and 5 of the 7 diagnosed later. Four of these 9 children with ocular involvement exhibited severely impaired vision in one eye, 3 being practically blind in one eye. After 20 years of follow-up 80% of the congenitally infected children had clinical manifestations.

1.3.3.3 RISK

Statistics can be used to quantify the risk of congenital toxoplasmosis. In the first place the incidence of an acquired infection depends on exposure to sources of infection and the proportion of the population that has not been immunized by previous infection: approximately 60% of pregnant women in the Netherlands.[102] As described above, toxoplasma infection of a pregnant woman does not always lead to congenital toxoplasmosis. *Toxoplasma gondii* is transmitted to the foetus in half of the cases, the transmission rate being 17% in the first trimester and increasing to 65% in the third trimester.[70,99] If the child is congenitally infected this probably will not be recognized at birth: about 70% of infected infants are asymptomatic.[79,80,84] Overt disease seems to be even more exceptional and is to be expected in only 10% of cases. Nevertheless the risk of sequelae due to congenital infection later on is very high, at least 80%,[81,86] and the pathobiology of *Toxoplasma gondii* indicates that morbidity will continue to increase with time.

1.4 LABORATORY TECHNIQUES FOR DEMONSTRATION OF TOXOPLASMA INFECTIONS

In view of the aspecific clinical picture of toxoplasma infection, laboratory support is needed for diagnosis. A broad variety of techniques is available: direct methods to look for the agent itself or products of its metabolism and indirect (mainly serological) methods to detect the agent by means of the immunoresponse; nevertheless the interpretation of test results may be hazardous. Demonstration of tachyzoites proves active infection while the demonstration of cysts, possible in all cases of latent infection, does not imply clinical disease. The same applies for the interpretation of serological tests. Since it is assumed that antibodies persist lifelong, their presence is

only indicative of the fact that infection occurred some time before. To diagnose an acquired infection seroconversion (transition from absence to presence of specific antibodies, i.e. from seronegative to seropositive) or a significant rise in antibody level must be demonstrated. At the moment that medical assistance is sought because of continuing aspecific complaints, the acute phase of the infection has already passed. For neonates the effect of prenatal symbiosis between mother and child must be taken into account: passively acquired maternal antibodies dominate the serological picture in the neonate.[103] In the case of ocular toxoplasmosis there is almost no effect of parasitic activity in the eye that can be measured in serum. To overcome these drawbacks various tests are used in parallel in order to characterize the process and establish a diagnosis.

1.4.1 DIRECT METHODS

Toxoplasma gondii can be demonstrated by histological techniques using autopsy material or patient specimens (e.g. cerebrospinal fluid, lymphatic node tissue, amnion fluid) and after inoculation of a specimen into mice or tissue culture. In addition to conventional staining techniques for histological examination (Giemsa and Kiewiet de Jonge) a direct immunofluorescent (DIF) method[104] can be used on unfixed specimens or a peroxidase-anti-peroxidase (PAP) method on fixed material.[105] For the mouse test the sample is homogenized and inoculated intraperitoneally into three mice. The presence of *Toxoplasma gondii* is indicated by the resulting active toxoplasmosis in the animals; the mice are bled after 6 weeks and the sera tested for a specific immunoresponse.
The drawback of this classical test to assess the presence of *Toxoplasma gondii* is the time-lapse before a definitive result is obtained.
Furthermore it does not distinguish between active and latent infections since inoculation of samples that contained cysts also gives a positive mouse test. It is imperative that the specimens be inoculated within a few hours of sampling,[106] otherwise tachyzoites will have died and secondary contamination of the material could also result in illness of the animals.
Isolation of parasites by means of a tissue culture technique yields results within a few days, a gain in time that is very important for antenatal diagnosis of congenital infection. In France, where prenatal diagnosis is based on foetal blood and amnion fluid samples, nine cases of congenital infection were diagnosed out of 107 patients who had serological evidence of acquired

toxoplasma infection during pregnancy:[107] isolation of *Toxoplasma gondii* by means of tissue culture of amnion fluid was successful in four cases, three of which were also detected by mouse inoculation; mouse inoculation alone was positive in another four cases; one case was detected by mouse inoculation of foetal blood.[108,109]
Experience with the detection of nucleic acids of *Toxoplasma gondii* in patient samples by means of gene amplification procedures, in particular the polymerase chain reaction (PCR), is encouraging and suggests that the PCR assay might be introduced for the diagnosis of toxoplasmosis in the not too distant future.[110-112]

1.4.2 INDIRECT METHODS

Toxoplasma can also be traced by specific antibodies. Several serological tests are available. Using different parasitic antigens they are used to characterize different phases of infection.[113] The classical Sabin Feldman (SF) dye test is still the test of reference.[3] Because of the risks for the laboratory staff of using live parasites, this test has been replaced by the indirect immunofluorescent (IIF) test and enzyme-linked immunosorbent assay (ELISA) for routine use. The simplicity of a direct agglutination test makes it valuable for large-scale screening programmes.[114-116]
Differentiation by antibody subclass might help to distinguish active from latent infection. This is based on the concept that IgM antibodies are produced soon after infection and disappear within several weeks, while IgG production has a longer lag time and antibodies of this subclass persist lifelong. Thus presence of IgM indicates that infection has recently been acquired. The use of very sensitive IgM tests has complicated the interpretation of serological results, because specific IgM antibodies classically considered to be characteristic of the acute phase of infection can be detected for a very long time after onset of the infection.[117-119]
Since, in contrast to IgG antibodies, IgM antibodies normally do not pass through the placenta, their presence in neonatal serum proves congenital infection of the infant. Nevertheless not all infected infants produce specific IgM. It appears that IgA, which behaves like IgM, is more likely to be produced by infected neonates.120 IgA methods are promising as a tool to differentiate between recent and latent phases of infection and will be of great help in diagnosing congenital infection.[120,121]

At the National Institute of Public Health and Environmental Protection (Rijksinstituut voor Volksgezondheid en Milieuhygiëne, RIVM), sera are tested with a so-called ELISA pentatest which assesses specific IgG and IgM but also specific free Toxoplasma antigen and circulating immunocomplexes of that antigen linked with IgG or IgM.[52] Antigens are released by proliferating parasites during the active phase of infection; they are measurable in tissues and blood, be it only for a short time since they are bound quickly to antibodies as the immunoresponse evolves. Thus in the subacute phase specific immunocomplexes can be determined. The test for circulating immunocomplexes may in fact be described as an amplification of the test for circulating antigens: parasitic (bound) antigens are detectable for a longer period. The presence of IgG complexes, in particular, is not necessarily specific for a recent infection; they can also be found in the case of relapse of a latent infection.[122] Perhaps unnecessarily, it must be stressed that quantitative assessment of antibodies is only worthwhile if more than one serum sample is available; a significant increase in antibody level points to a recent infection. No conclusion may be drawn from a high level of antibodies in one sample:[123] the titre provides more information about a person's immunoresponsiveness than the time lapsed since infection occurred.
A Western blot technique that is available in reference laboratories is used to diagnose congenital infection. This method discriminates antibodies against different antigenic determinants of the parasite by a gel electrophoretic procedure. Thus the antibody patterns of blood samples from a mother and her child, who is suspected of having congenital toxoplasma infection, can be compared.[124,125] A difference between mother and child, especially if the child shows components that are not observed in the mother's sample, indicates that antibodies that cannot be acquired passively are present.
The occurrence of "natural antibodies" must be mentioned. These antibodies do react with *Toxoplasma gondii* and may cause a positive IgM test that is, however, not indicative of a history of toxoplasma infection.[126-128] Natural antibodies can be assessed by indirect immunofluorescent assays where a polar staining of parasites, know as the capping phenomenon, is seen.

1.4.3 DIAGNOSIS VERSUS SCREENING

Moving from curative to preventive medicine, diagnostic techniques are being applied increasingly for screening purposes. Whereas a laboratory test can

be a very valuable support of a diagnosis that is suspected on the basis of clinical observations, the same test may cause confusion when used for a healthy person. In the latter case more significance may be attached to a result than would ever be the case for a diagnosis which is always a compilation of clinical and laboratory examinations. False conclusions may be drawn.
Diagnosis and screening have different purposes: diagnosis aims to confirm a suspected illness in an individual who often needs to be treated; screening focusses on the recognition -at the population level- of risk groups that could benefit from health services, such as preventive programmes.
Diagnosis and screening therefore have different characteristics: to reach a diagnosis several samples from one person are tested quantitatively using a number of assays; for screening purposes a single sample is usually tested with one assay that is simple, rapid, cheap and applicable on a large scale, thus one that can be automated. The screening test is a qualitative one, serving only to assess the presence or absence of a condition. Screening tests are widely abused since interpretation of their results is often extended to a diagnosis.[129] With respect to toxoplasmosis, screening tests may be used to assess the immunity of women during pregnancy: a negative test indicating no previous infection or susceptibility, a positive test indicating previous infection or non-susceptibility. If screening is repeated at various intervals, there is a transition from the population level to the individual level: a change from seronegative to seropositive points to infection which has to be confirmed by further quantitative testing. A very good diagnostic test is one with a high specificity and a high sensitivity; however this will not always be good enough for screening purposes because it does not guarantee a high probability of disease for those with a positive test.[130] The results of testing are markedly influenced by prevalence of the disease: if the disease is rare, the test will produce a lot of false-positive results.[56]

1.5 THERAPY

Unfortunately the parasites hidden in tissue cysts are insensitive to any drug.[131] Actively reproducing tachyzoites, however, can be attacked by chemotherapeutics that interfere with their metabolism. As a consequence it is not possible to eliminate a latent infection. Treatment during the acute phase of infection can combat the parasite and will be beneficial if the infection has not yet progressed to the stage of irreversible tissue damage.

1.5.1 FOLIC ACID ANTAGONISTS

Pyrimethamine (Daraprim®), known as an anti-malaria drug, is a folic acid antagonist that inhibits replication of parasites by impairing DNA synthesis. Its effect in mice was demonstrated in the fifties[132] and the drug was subsequently applied for treatment of toxoplasma infections in man. But its action is also noticeable in host tissues with a high cell turnover, where it has a damaging effect, for example bone marrow depletion. For the same reason teratogenic effects of the drug are to be expected when administered during pregnancy, which thus was thought to be contra-indicated.[133] This risk was observed in experiments in rats but the production of malformations could be prevented by concomitant administration of folinic acid, which man but not the parasite can utilize.
In man only one single case of malformation has been reported[134] and the relation to pyrimethamine was later questioned.[135] The drug has been used all over the world against malaria for more than 30 years without any evidence of teratogenicity. In addition no malformation was observed in a careful study of children born to women with toxoplasmosis who received very high doses of pyrimethamine during the first trimester.[136] Based on these data WHO in 1978 approved the free use of pyrimethamine for malaria prophylaxis during pregnancy. Pyrimethamine together with folinic acid (Leucovorin®) is prescribed for acquired toxoplasma infection during pregnancy after more than 20 weeks of gestation in Austria and in the case of proven foetal infection in France.
The drug may also cause gastrointestinal complaints, headache and a bad taste in the mouth.

Sulfonamides, which as antagonists of p-aminobenzoic acid also interfere with DNA synthesis, are active against *Toxoplasma gondii*; especially sulfadiazine, sulfamethazine and sulfamerazine appear to be effective.[137] Sulfonamides bind to foetal protein; after birth this may result in a high concentration of free bilirubin that the neonate is unable to detoxify. For this reason it is advised not to use sulfonamides after 36 weeks of gestation.

Of great importance is the synergistic action between sulfonamides and pyrimethamine, as first demonstrated in a mouse model;[137] given together they induced more than six times the effect expected of the additive action.[138] The most likely explanation of this pronounced synergism is that sequential blocks are produced along a single important metabolic pathway.

The first reports on combined administration in man, reviewed by Eyles, were very encouraging and the combination nowadays is the regimen of choice for treatment of toxoplasma infections (except in case of pregnancy).[139]

1.5.2 SPIRAMYCIN

Spiramycin (Rovamycin®) is a macrolide antibiotic derived from *Streptomyces ambofaciens* which has been shown to be active against *Toxoplasma gondii*.[140,141] It reaches a high concentration in the placenta, making it very useful for the prevention of vertical transmission of a toxoplasma infection.[142] Transmission of parasites from mother to foetus is delayed in the placenta, which explains why treatment may still be effective even if there was some (nearly inevitable) delay in diagnosing the maternal infection. Moreover it is suggested that the placenta behaves like a relay for Toxoplasma and that, even in the case of maternal infection early in pregnancy, parasites may be transmitted to the foetus during labour.[70] For this reason, in France the therapeutic regimen for infection acquired during pregnancy now includes 3 grams of spiramycin daily, to be continued up to the end of pregnancy.[107] There is no free passage of the drug through the placenta; if foetal infection has already occurred, it will have no effect.[143] Adverse reactions were not reported.

1.5.3 TREATMENT OF ACQUIRED TOXOPLASMA INFECTION

An acquired toxoplasma infection is a self-limiting disease, generally without any symptoms. Treatment of adults is hardly ever necessary. In the case of severe symptoms due to an active infection, the combination of pyrimethamine (Daraprim®)(loading dose 100 mg/day in 2 doses, later 25 mg/day in 2 doses), sulfadiazine (4 g/day in 2 doses) and folinic acid (Leucovorin®)(5 mg twice a week i.m.) is advised.
Prescription of drugs for an acquired toxoplasma infection during pregnancy as a preventive measure has been studied since the sixties. The administration of any drug during pregnancy must be based on a strict, carefully considered indication. The potential danger is best illustrated by the teratogenic effect of pyrimethamine in mice. This drug should never be given to prevent infection of the foetus; if foetal infection has, however, already occurred and pregnancy has lasted over 20 weeks, the drug is prescribed as therapy

for the foetus.[107,144] Limitations of prenatal treatment with the sulfadiazine-pyrimethamine combination have been reported[145] but were to be expected due to inevitable diagnostic delay.
Controlled studies are needed to determine whether the potential risks of treating the mother with pyrimethamine are warranted by improved foetal outcome. Other less toxic possibilities for preventive treatment of infected pregnant women are being considered. Several studies performed, especially in France, indicate that spiramycin treatment (1 g 3x daily) considerably reduces the transmission rate.[79,80,99,143,146] Often sulfadiazine (1 g 3x daily) is added. The effect of preventive treatment on transmission of parasites to the foetus is now thought to equal a 50% reduction. Therapy generally was prescribed for three weeks but its administration now is being continued until the end of pregnancy.
The possibility of changes in the clinical spectrum was first suggested by Desmonts and Couvreur;[146] they found 23% infected children when the mother was treated versus 61% in the untreated group. There was no difference in the number of mild and subclinical infections; however, the number of stillborns decreased and the number of severe cases slightly increased in the treated group. They stated that treatment might sometimes have prevented foetal death but was undertaken too late to prevent severe damage. In later extended studies, however, this has been refuted.[147,148] Many studies on maternal treatment compare the outcome with historic data; none of the studies includes appropriate controls. In a commentary on such a study performed in France,[149] it was emphasized that the improved outcome of maternal treatment can be attributed in part to the early diagnosis of foetal infection in France and consequent selective termination of pregnancies with severely damaged foetuses.[150]

1.5.4 TREATMENT OF CONGENITAL INFECTION

The rationale for treatment of congenital toxoplasma infections is dualistic: it may be therapeutic in the case of toxoplasmosis and it may be prophylactic in the case of toxoplasma infection, thus aiming to prevent the development of illness.
Since the available drugs do not act against latent infections, the decision to treat should be founded on assessment of the activity of infection, thus justifying aggressive therapy, and not on the severity of the presented symptoms. The same restraints are recommended for prophylactic treatment.

A majority of lesions not yet seen at birth become manifest during the first year of life.[151] Relapse may occur anytime throughout life, among toddlers and during puberty. Should prophylactic treatment then be continued throughout life? In anticipation of the waning maternal immunity, which is thought to account for this relatively high incidence of new lesions during infancy, many authors recommend treatment during the first year of life. The effect, however, of this regimen has never been evaluated in a well-controlled study. Moreover cellular immunoresponse plays a major role in the defense against *Toxoplasma gondii*.
At present a multicentre randomized study is in progress in the USA to evaluate this prophylactic treatment.[152]
The accepted regimen for therapy for neonates is as follows: The combination of pyrimethamine (1 mg/kg/day, as one oral dose every 3 days) and sulfadiazine (100 mg/kg/day in 2 oral doses) gives the best results;[148] in addition 5 mg folinic acid must be given twice a week intramuscularly to prevent toxicity. During the first year of life 3 to 4 21-day courses are prescribed, alternated with a course of 100 mg spiramycin/kg/day in 2 doses for 30 to 40 days. French authors recommend this regimen for both overt and subclinical infections. In the case of inflammatory signs, for example in fundo or high protein concentration in cerebrospinal fluid, corticosteroid therapy (prednisone 1-2 mg/kg/day in 2 doses) is added. In the case of suspected infection, i.e. maternal infection without a definite diagnosis for the child, one course of pyrimethamine/sulfadiazine followed by spiramycin or spiramycin for 45 days is recommended.[143,153]

1.5.5 TREATMENT OF OCULAR TOXOPLASMOSIS

The same considerations apply for the treatment of ocular toxoplasmosis. If a new fulminating lesion is recognized the pyrimethamine/sulfadiazine combination may be effective, but it is toxic. Despite administration of folinic acid, frequent haematologic adverse reactions have been reported.[154] Several drugs are considered less toxic, including clindamycin and trimethoprim with sulfamethoxazole, but have not proved effective in limiting the duration of inflammatory activity.[154]
With the available drugs, therapeutic intervention to halt chorioretinitis – which in principle is a self-limiting disease – is only recommended in the case of impairment of the papilla or the macula.

1.6 PREVENTION

The best approach to control of congenital toxoplasmosis is prevention, which has to be aimed first and foremost toward (future) pregnant women. Primary prevention comprises all measures taken to prevent primary infection. Vaccination of girls in order to induce immunity artificially before pregnancy is not possible because no vaccine is available. Primary prevention relies on information and education of women about how they can avoid exposure to toxoplasma by adaptation of their behaviour.[15] The consumption of meat that has not been heated enough is strictly forbidden.
Direct or indirect exposure to cat faeces, that might contain oocysts, must be avoided: the cat litter must be cleaned daily (preferably not by the woman herself but if this is inevitable she must wear gloves) so that oocysts, if present, will be removed before sporulation; there is no need to get rid of cats if the proper hygienic measures are taken;[155] because of the risk of picking up oocysts indirectly from the environment, vegetables that might be contaminated must be washed thoroughly and gloves must be worn for gardening. Primary prevention is feasible, but there are no definite figures that quantify the effect: some state that it might reach 100%,[156] others expect an effectivity of 50%.[157] If primary prevention is not sufficient, there remains the possibility of secondary prevention. This strategy is aimed at blocking vertical transmission of parasites from the infected mother to her unborn child by treatment of the mother. Since the maternal infection is usually asymptomatic it must be traced by specific measures: repeated serological screening of women at risk.

1.7 SITUATION IN EUROPEAN COUNTRIES

The World Health Organization has urged European countries to consider the possibilities of the introduction of a preventive programme for pregnant woman. WHO meetings on public health aspects of toxoplasmosis were held in Graz (Austria) in 1984 and in Hannover (Federal Republic of Germany) in 1987. Since then international cooperation has been encouraged by the installation of study groups that coordinate investigations and develop strategies. The information in the next section is for the most part derived from the proceedings of these WHO meetings.
Two European countries have a systematic screening programme that is regulated by law: France and Austria.

FRANCE

In France screening for the presence of specific antibodies is started at a mandatory prenuptial examination and repeated on a voluntary basis for initially seronegative women every month. This unstandardized procedure has been widely used in France since the sixties and was included in an obligatory programme in 1978.[158] The costs of serological surveillance are covered by the social security system. It seems that the emphasis actually lies on secondary prevention. Notwithstanding the compulsory serological screening, only 84% of women questioned during a survey in 1986 were aware of the consequences of a toxoplasma infection during pregnancy; 30% did not know any of the preventive measures. The need for information is clear, and a leaflet is being prepared and was expected to be distributed in 1990. The overall seroprevalence among pregnant women in France is estimated to have been 65% in 1988, the seroconversion rate for seronegative women 0.4-1.6%.

The force of infection is thought to be unchanged since 1961.[159]

AUSTRIA

In Austria the first blood sample is taken at the beginning of pregnancy and repeated, in the event of a seronegative result, in the second and third trimesters.[160,161] Those who show antibodies early in pregnancy are retested after 3 weeks in order to exclude the possibility of a recently acquired infection. Since the introduction of obligatory toxoplasmosis surveillance in Austria no case of congenital toxoplasmosis has been observed among children of women who were tested in accordance with the directives and received timely treatment in the case of primary infection.

The very few cases of congenital toxoplasmosis that have occurred involved exclusively children of women who had not been screened or were inadequately treated. The Austrians claim that the frequency of congenital toxoplasmosis in their country has decreased from about 0.7% to below 0.1% since the programme was introduced in 1975.

OTHER COUNTRIES

Screening of pregnant women was evaluated in the seventies in Norway and the investigators advised the health authorities to postpone obligatory surveillance until more knowledge was gained about the effect of treatment and better serological tests became available.[162]

Introduction of a preventive programme in the former Federal Republic of Germany is under discussion. It has been emphasized that the availability

of standardized serological techniques throughout the country is a prerequisite. In the former German Democratic Republic there is no nationwide programme but screening is applied in several districts. The same applies for Belgium where screening is common in the University Hospitals. In Sweden attempts to provide information on primary preventive measures are made. In the United Kingdom no consensus exists on the desirability of a screening programme.

1.8 SITUATION IN THE NETHERLANDS

In 1978 the then Undersecretary of Public Health and Environmental Protection asked the Health Council (Gezondheidsraad, a scientific advisory board for the Minister and Undersecretary) for advice on the desirability of screening for toxoplasmosis in neonates. In 1980 the chairman of the Health Council installed a committee to study this issue; at the end of 1983 this committee completed its report: "Advice on the subject of detection of congenital toxoplasmosis".[102] The committee suggested that "a study be made of the occurrence and possibilities of prevention of congenital toxoplasmosis. After 1 to 2 years recommendations can be made on the desirability of a national screening programme, based on the results of the trial study. Given the uncertainty of the effect of treatment of recently infected pregnant women on the incidence of congenital toxoplasmosis as well as the unfavourable cost/benefit analysis obtained for this service, the committee wishes the trial to be confined to the effect of primary prevention on the incidence of congenital toxoplasmosis, at first". Subsequently, the Medical Office of Health (Geneeskundige Hoofdinspectie, GHI) called upon Prof.Dr. J. Huisman, then head of the Department of Infectious Diseases of the Municipal Medical and Health Service (Gemeentelijke Geneeskundige en Gezondheidsdienst, GG&GD) of the city of Rotterdam, to explore the possibilities of conducting such a trial study in the province of South Holland. A group of delegates from the Netherlands Institute for Preventive Health Care (Nederlands Instituut voor Preventieve Gezondheidszorg, NIPG: H.J. Spook †, Dr. G.J. Vaandrager, W.J. Meijer) and the National Institute of Public Health and Environmental Protection (Rijksinstituut voor Volkgezondheid en Milieuhygiëne, RIVM: Dr. F. van Knapen, M.A.E. Conyn-van Spaendonck) started to prepare a definite proposal for the study. At the beginning the group considered a study in three consecutive phases:
1. baseline assessment

2. evaluation of primary prevention
3. evaluation of secondary prevention.

Some considerations and events that have determined the ultimate design will be discussed briefly in the following section.

1. BASELINE ASSESSMENT

The Health Council proposed to "assess the incidence of congenital toxoplasmosis in a certain region by serological examination of newborn children. This study could be carried out in parallel with the preventive study of pregnant women. This approach is judged important because of possible alterations in the epidemiology". It is known that no adequate parameter is available that proves or excludes congenital infection of a neonate definitely at birth. However, the frequency of congenital toxoplasma infections in a cohort of newborns is reflected by the prevalence of specific IgM antibodies. The assessment of the prevalence of IgM antibodies in a cohort of neonates was considered to be appropriate for epidemiological purposes.

2. EVALUATION OF PRIMARY PREVENTION

A proposal for a scientific trial to investigate the effect of primary prevention was made by the Department of Health Education (Gezondheidsvoorlichting en Opvoeding, GVO) of the Municipal Medical and Health Service (Gemeentelijke Geneeskundige en Gezondheidsdienst, GG&GD) of the city of Rotterdam (Dr. W.F.M. de Haes) together with the RIVM and the Department of Health Education of the Provincial Home Nursing Organization (Provinciale Kruisvereniging, PKV) South Holland (Mrs. Ronner). The study should answer the following questions:

- What is the effect of health education on the prevention of congenital toxoplasmosis?
- What is the best way to provide the information and what are the necessary provisions, means and organizational conditions?
- What instruments can be used to assess the effect?
- What kind of resistance will be encountered in the target population?
- Do the pregnant women and family members experience the desired adaptation of their behaviour as a burden?
- Which aspects should be included in a cost/benefit analysis?

If primary prevention should become a programme for the whole population, health education could possibly be the cheapest approach to intervention, but evaluation of the effect is time and cost-consuming.

3. EVALUATION OF SECONDARY PREVENTION

In accordance with the advice of the Health Council, initiation of a study on the effects of secondary prevention was conditional on the effects of primary prevention, as measured in the previous study.

On request of the intended financier (Praeventiefonds, Prevention Fund), a proposal for the third phase of the study was submitted simultaneously with the proposals for the first two phases. In consultation with the Prevention Fund, the Medical Office of Health (GHI), and the National Institute of Public Health and Environmental Protection (RIVM), it was decided subsequently that primary and secondary prevention should be combined into one study. Thus the aim of the Health Council, i.e. to attack the toxoplasmosis problem first from the "non-medical" standpoint, was abandoned. The investigators emphasized that later on a conclusion could be drawn only on the combined effect of primary and secondary prevention. In the meantime screening during pregnancy was being applied increasingly on an individual basis in prenatal management all over the country. The Laboratory of Parasitology and Mycology of the RIVM was consulted more frequently when problems arose about the interpretation of results of serological examinations performed early in pregnancy. The need for a trial study from which directives and conditions for a preventive programme could be derived became increasingly evident. After some delay it was decided in 1986 that a preventive study should be carried out. The RIVM was given full responsibility by the GHI. The study acquired a more pragmatic character.
In addition to confirmation of the effect of preventive measures, the development of a suitable and standardized programme became a major aim of the study. The study was performed in the province of South Holland. The ultimate design of the study will be described in chapter 3.

2 BASELINE STUDY

2.1 INTRODUCTION

In 1983 the Dutch Health Council estimated the number of congenitally infected children in the Netherlands: every year 800 children were expected to be born with a toxoplasma infection.[102] This figure is derived from Dutch seroprevalence data, on the one hand,[71] and information about the risk of transmission of parasites from mother to foetus provided by French studies, on the other.[146] Only some of these congenital toxoplasma infections (CTI) can be recognized at birth on the basis of clinical manifestations: congenital toxoplasmosis (CT).

In the Netherlands a systematic study to estimate the frequency of congenital toxoplasma infections had never been performed. Therefore the Health Council recommended that the "incidence of congenital toxoplasmosis be determined by serological examination of neonates" as a part of a preventive study in the province of South Holland.

Some critical remarks on this item from the report of the Health Council can be made.

If parasites pass through the placenta following primary infection of a woman infection of the foetus occurs. This new case of congenital toxoplasma infection (incidence) can only be recognized in the antenatal period by invasive techniques (amniocentesis, foetal blood sampling by puncture of the umbilical vein).[107] It is clear that the use of these techniques is not appropriate as a tool for epidemiological research.

Assessment of the incidence of congenital infection has to be postponed until birth; as a matter of fact, at that moment the occurrence of CTI is assessed on the basis of prevalent cases, namely cases that have already existed for some time and which can be detected by the presence of specific antibodies. One should be prepared for some pitfalls inherent in serological examination of neonates. The production of antibodies against *Toxoplasma gondii* by the neonate indicates infection, as the immune system must have

been stimulated by parasites. The mere presence of specific IgG, however, is not decisive in this respect since these antibodies are most likely to be antibodies of maternal origin that have passed through the placenta. If a congenitally infected child is already producing antibodies at birth, it probably will not be possible to differentiate these antibodies from maternal antibodies unless specialized techniques, such as Western blotting, are used.[125] During the first months of life, however, the maternal antibodies gradually disappear and the antibodies produced by the child begin to dominate until they determine the serological status in the second half year. At birth, therefore, specific IgM should be looked for as a sign of infection of the neonate. Maternal IgM antibodies normally do not pass through the placenta so that the presence of IgM in neonatal serum indicates antibody production induced by the child itself.[163-167] Placental leakage is reported to occur in 10-60% of cases.[168,169] Absence of specific IgM, however, does not rule out the possibility of infection;[170] even children with clinically manifest toxoplasmosis often do not produce IgM.[34,84] Struck, on the other hand, hypothesizes that especially in cases of infection without detectable IgM, severe symptoms are to be expected due to a very early foetal infection (before 20 weeks of gestation).
Immunotolerance of the foetus in this period could explain the absence of IgM.[171]
Like IgM IgA antibodies are unable to pass through the placental barrier, and recent reports suggest that detection of specific IgA can be of additional value in the recognition of congenital infection.[119,121,172] From these considerations of the serological response of congenitally infected children, it follows that the incidence of congenital toxoplasma infections (CTI) can at best be derived from the prevalence of specific antibodies at the end of the first year of life. In doing so we assume that the risk of acquiring primary infection during the first year of life is small. Loss from the cohort due to death from clinical toxoplasmosis has to be taken into account.
The idea is that, for epidemiological purposes, the prevalence of specific IgM in neonates can be used as an indicator of the occurrence of CTI in a cohort, especially if IgM seroprevalence in successive cohorts is measured; but it is impossible to calculate the true frequency of CTI from this number.

2.2 IGM SEROPREVALENCE IN A COHORT OF NEONATES

The actual baseline study consisted of the assessment of IgM prevalence

in a cohort of neonates in order to estimate the frequency of congenital toxoplasmosis, the intention being to use this frequency as a parameter for evaluation of the effect of preventive measures that were to be introduced later on. The study was planned to last one year, but the possibility that the baseline study would have to be prolonged if not enough IgM-positive neonates could be traced within one year was considered.
Nearly all the children born in the Netherlands are included in the phenylketonuria (PKU) and congenital hypothyroidism (CHT) screening programme.
The Dutch Health Council suggested using this material for the baseline assessment of congenital toxoplasma infections. This was an efficient and cheap way to obtain blood samples, eliminating the need for vein puncture, subsequent processing of serum and space-consuming refrigerated storage. The possible use of the filtre paper samples of the PKU/CHT screening programme for this study was discussed with the National Steering Committees of the PKU/CHT programme. As a rule (98.66% of cases) only three-quarters of the available material is used for PKU and CHT tests; one-quarter is left over.[173] The committees allowed us to use the rest of the filtre paper samples, provided absolute anonymity was maintained.

2.3 MATERIALS AND METHODS

For the PKU/CHT programme, capillary blood is sampled by heel puncture on day 5-8 after birth (the cumulative percentage of participation on the 9th day is 75.7%); the blood is absorbed onto filtre paper (Sleicher and Schulz 2992) in a volume sufficient to saturate the area within a preprinted circle with a diameter of 9 mm. Participation in this programme is 99.58% of all children born alive, or 99.76% if children who die before the age of screening are excluded.
During 1986 and part of 1987, the PKU/CHT laboratories in Rotterdam, Amsterdam and Bilthoven sent all material remaining after they had finished the tests to RIVM once a month. For a few children (1.34%) no sample was available because the results of PKU or CHT screening required an additional test.[173] It is not certain that the samples were always kept under similar storage conditions until forwarded. When received by RIVM, the materials were stored at 4°C in plastic bags.
The procedure for elution of the filtre paper samples and the serological examination of the eluates were assessed in an extensive preliminary investigation.[174]

The eluates of 32,000 filtre papers (24,000 children born in 1986, 8,000 children born in 1987) were examined early in 1987 with an ELISA for specific IgM.[175,176]

2.4 RESULTS

The ELISA for specific IgM in 32,000 filtre paper samples yielded a positive result 25 times. IgM seroprevalence, which was similar for the 1986 and the 1987 samples, amounted to 0.08%.

2.5 DISCUSSION

What is the significance of the low IgM prevalence among neonates? Different situations might be indicated by a negative test result:
- there is no toxoplasma infection;
- the child is infected, but there is no specific IgM in the circulation at the time of examination;
- the child is infected and produces IgM antibodies but they are not detectable with the test used.

In the early sixties the first reports on antibody production by the foetus were published.[177] Eichenwald was able to assess specific IgM produced by infected infants born as early as the twenty-eighth week of gestation.[178] Several reports suggested that IgM can be used to look for infected neonates; but these earlier reports are based on the test results for a highly selected group of patients.[163,165] Later on Remington et al. pointed out the possibility that some infected infants may not have a demonstrable IgM antibody response; they stated that there is no relation with early treatment of the infected child nor with the severity of disease: different serological patterns may be observed in infants with asymptomatic infection as well those with clinical signs.[165] Araujo's work on an experimental rabbit model supports the concept that maternally transmitted IgG may suppress the IgM antibody response in the foetus and newborn infant.[164] None of 54 children at risk for congenital toxoplasma infection, identified in a prospective study of pregnant women in Norway, showed IgM -not even the 3 children that proved to be infected.[179] In a prospective study we found IgM in cord serum from 1 out of 12 congenitally infected children (see chapter 5). A positive result of the IgM assay is, of course, valuable for detection of a congenital toxoplasma infection,

although the titre may be rather low.[180] But for a careful interpretation of the test results it is also necessary to exclude false-positive reactions due to disturbing factors, such as rheumatoid factors.[181] The sensitivity of different assays that can be used to determine IgM differs considerably.[182] From our experience with the detection of specific antibodies against *Toxoplasma gondii* in blood samples on filtre paper, such as those used for the biochemical screening for PKU and CHT in the Netherlands, we concluded in a preliminary study that the method is not appropriate for delayed assessment of IgG antibodies: comparison of the IgG results of the ELISA for sera and filtre paper samples indicated that the latter were not accurate. It was mainly the preservation time that had a negative influence on the reliability of the test: an unacceptable drop in specificity as well as lack of accordance between multiple simultaneous assays of samples from one person. The system was sufficiently reliable for IgM detection. We could detect the signal of the presence of IgM in eluate samples with our ELISA test system even after a long preservation period; furthermore no false-positive results were recorded in an experiment with 1000 neonates.[174]

If the incidence of congenital infections is assumed to be 0.5%[102] and specific IgM is detected in only 25% of infected neonates,[70] then the expected prevalence of IgM would be 0.125%, which is over one and a half times the value of 0.08% found in this study. Assuming that the measured prevalence gives a good indication of the true prevalence of IgM antibodies among a group of neonates, the question arises whether the calculations in the report of the Health Council are realistic. A large-scale prospective study of the incidence should be done to verify the estimates of the expected number of infections during pregnancy while follow-up studies of children of infected mothers are needed to assess the risk of congenital infection.

In March 1987 systematic measures to prevent congenital toxoplasma infections were introduced in the province of South Holland. If this should result in a decline of the number of congenital infections, a drop in the IgM prevalence might be expected. To demonstrate a relation between the reduction of IgM prevalence and the application of preventive measures, a minimum number of positive findings during the baseline study was required.
We assumed that during a one-year baseline study of 30,000 neonates an IgM prevalence of 0.5% would be observed; it would then be possible (at a confidence level of 95%; $\alpha = 0.05$) to relate a 50% reduction of this prevalence

(i.e to 0.25%) in a cohort of 30,000 observed during one year, to the influence of preventive measures (power analysis). However, in our baseline study a prevalence of only 0.08% was found. It was not possible to prolong the baseline study since the intervention study had already begun in South Holland. With such a low prevalence, on the other hand, it would have been necessary to expand the intervention group four times in order to reach significance; in other words, it would be necessary to extend the number of persons enrolled in the intervention study to 120,000, either by prolongation of the study period or by enlarging the study region to include other provinces because the expected number of births in the province of South Holland was approximately 38,000 a year. Having already faced many practical problems in the implementation of the study, further examination of filtre paper samples was abandoned.

2.6 SUMMARY AND CONCLUSIONS

Baseline assessment of IgM prevalence was started in order to obtain a parameter for evaluation of the influence of preventive measures on the occurrence of congenital toxoplasma infections. It was believed that IgM prevalence could be used for epidemiological purposes, although it was clear that IgM may only be found in a small number of congenitally infected infants and there is no known relationship to calculate the true number of infections from the determined prevalence. An IgM prevalence of 0.08% was found for 32,000 neonates. This very low prevalence led us to reconsider the usefulness of the IgM prevalence. IgM is a valuable tool for the diagnosis of congenital infection in individual cases, and the prevalence of IgM in a population of neonates reflects the degree of congenital infection in the cohort. However, a parameter of such low prevalence cannot be used to evaluate statistically the effect of prevention on the occurrence of congenital infection within a reasonably sized cohort. We decided, therefore, that the prevalence of specific IgM antibodies against *Toxoplasma gondii*, as observed in this study, was far too low to function as a useful indicator of changes in the occurrence of congenital toxoplasma infections.

3 INTERVENTION STUDY: PRIMARY AND SECONDARY PREVENTION

3.1 INTRODUCTION

Congenital toxoplasma infections can be avoided by primary preventive measures that reduce the risk of primary infection in pregnant women and secondary preventive measures that lead to early detection of such an infection in order to treat it, thus diminishing the risk of infection of the foetus. Although the Dutch Health Council planned an extensive evaluation study to measure and compare the effects of each strategy separately, it was not possible to realize such a study as explained in section 1.8. Eventually a combination of strategies was introduced in a study designed to establish the effects of prevention, although there could be no definite conclusions on the effect of primary prevention, on the one hand, and the effect of secondary prevention, on the other. Furthermore it was essential that standard operational procedures be followed and the logistical problems encountered in a study on this scale be evaluated in order to establish the conditions for such a programme for the whole country. A feasible and standardized programme would subsequently be derived from the combined experience of a large group of involved health care workers.
The outline, methods and materials of the intervention study are described in this chapter.

3.2 DESIGN AND ORGANIZATION

Primary prevention consisted of thoroughly informing (future) pregnant women about the risk of infection and how to avoid it: do not consume raw meat and avoid contact with cat faeces: clean the cat's box daily (it is better that she not do this herself), wear gloves when gardening, wash fresh vegetables thoroughly.
Secondary prevention consisted of repeated serological examination to reveal

primary infections; once an infection was recognized, therapy was initiated immediately in order to prevent transmission of parasites from the infected woman to her foetus.

Assuming a risk of toxoplasma infection during pregnancy of 12 cases per 1000 women, a large number of subjects had to participate in the preventive study. The necessary number of participants was approximately 30,000 in one year, as advised by the Health Council. The province of South Holland was chosen as study region because there are approximately 38,000 births a year.
Enrollment in the study was planned to last one year; from March 1, 1987, to March 1, 1988. The study was called the TIP study: toxoplasma infection prevention.
Prenatal care in the Netherlands is performed by midwives, general practitioners and obstetricians (M/GP/O). An important part of the study was carried out by thess groups because they have direct contact with pregnant women; their task was education of the women and collection of the blood samples. For reasons of standardization serological testing could not be performed in a large number of laboratories. It could have been restricted to one laboratory in the study region, while the RIVM was responsible only for coordination and evaluation of the study and the necessary reference assays. Because the costs became too high for a local laboratory, the RIVM finally also took charge of the routine screening tests. The different laboratory tasks were executed in different units: the screening tests in the so-called TIP laboratory, the reference tests in the laboratory for parasitology.
The study was set up in close consultation with the different professional groups concerned and the Chief Medical Officer of Health.

In January, 1986, the Chief Medical Officer of Health sent a request for cooperation and support to midwives, general practitioners and obstetricians in the study region. Those interested could reply via an enclosed form and subsequently received additional information on the problem of congenital toxoplasmosis, an extensive outline of the study and materials for the instruction of women together with participation forms, needles and syringes for blood sampling and mailing boxes to return the samples. Moreover, lectures were presented to the involved professional groups in South Holland whenever requested. During the study it was always possible to ask about individual cases; the RIVM, however, did not inform the pregnant women about an eventual infection because their doctors and midwives were supposed to support and advise them personally.

The study in South Holland, which was announced to the public in national and regional journals, received considerable attention from the media.

3.2.1 INFORMATION AND EDUCATION

Pregnant women consulting a midwife or physician were informed about the aim and consequences of the study at their first visit. The problem of congenital toxoplasmosis had to be explained and the simple measures recommended to minimize the risk of infection had to be described. To support this verbal instruction the RIVM provided a leaflet with information on both primary and secondary prevention. A reprint of the brochure can be found in appendix 1a. Depending on the results of the first blood test, the need to follow the preventive measures was stressed again and repeated at every visit.

3.2.2 SCREENING OF BLOOD SAMPLES

The TIP study was initiated as a preventive study: its purpose was to identify those women who have never been infected with *Toxoplasma gondii* and thus could profit from the proposed preventive measures. The tool used to identify infected women was a serological test (enzyme-linked immunosorbent assay, ELISA) that assesses the presence or absence of specific IgG antibodies against Toxoplasma. A woman lacking these antibodies is unlikely ever to have been infected, although there is a theoretical possibility that infection has just occurred and that antibodies have not yet been produced in detectable quantities. This, however, will be very rare. Moreover there is no unambiguous method to determine recent infection retrospectively in a sample taken at the beginning of pregnancy: neither the presence of IgM nor the level of IgG antibodies in one sample is a sufficiently accurate parameter to decide whether infection has occurred within the preceding 2 to 3 months. As a result we did not attempt to establish a retrospective diagnosis. This issue will be discussed in detail in chapter 7.
A blood sample was taken from the women who were willing to participate. If the test result was positive, i.e. antibodies indicative of earlier infection were detected, the woman was told at her next visit that she was not at risk and that there would be no further follow-up. If, however, no antibodies were found, blood samples were tested according to a schedule proposed in the study protocol.

The efficacy of treatment of the infected mother in the prevention of congenital infection depends on the time lapsed since infection occurred and thus on the interval between consecutive samples. One has to choose a workable schedule on arbitrary grounds, taking both the acceptable load for pregnant women, from the practical as well as psychological standpoint, and the acceptable workload for the health workers concerned into account. When classical seroconversion is observed it might be too late: the parasites may have had time to elicit the immunoresponse, maybe also to pass through the placenta.
We advised sampling as soon as possible after pregnancy was established and at 18, 24 and 32 weeks of gestation; a cord sample of the neonate should also be tested; finally it was recommended that a baseline sample should be obtained from women who expressed the wish to become pregnant soon. Table 3.1 shows the schedule used to determine the actual testing dates. Guided by the period of gestation at the time of the current sample, the week in which the next sample should be taken can be read from the table. The result was translated into a target date for each individual woman as follows:

$$td = lp + (7 \times tg)$$

where td is the target date, lp is the first day of the last menstruation period and tg is the target gestation period in weeks. This target date was listed in the letter sent to the patient's M/GP/O to inform her/him about the last test result; a form for submission of the next sample was enclosed (see addendum).

The M/GP/O could use the target date as a guideline since usually the frequency of antenatal control visits does not parallel this schedule; it was recommended that sampling be performed within a period of four weeks around the target date but not within a month of the previous one. As can be seen in table 3.1, a sample was also planned at 36 weeks of gestation if the sample intended to be taken at 32 weeks was taken so soon that the period up to parturition would have been too long.

Table 3.1 Schedule for repeated testing: target gestation period (weeks) at the time of the next sample guided by gestation at the time of the current sample.

gestation period in weeks	
current sample taken at	next sample to be taken at
0*-13	18
14-19	24
20-27	32
28-31	36
32-	cord sample at birth

* For a woman not yet pregnant at first sampling, it was recommended that the next sample be taken as soon as she knew she was pregnant.

3.2.3 DIAGNOSIS AND THERAPEUTIC INTERVENTION

A seropositive result for a woman who was seronegative at the previous test gave rise to suspicion; such a seroconversion pointed to infection during the intervening period, but this had to be confirmed by simultaneous testing of two successive samples. Initially the screening samples were only tested qualitatively; additional diagnostic testing of the pair of samples also allowed quantitative interpretation of the serology. A primary infection was proven by the demonstration of seroconversion, a change from seronegative to seropositive, by means of this simultaneous test.
Once the diagnosis was confirmed, the doctor or midwife concerned was informed immediately by telephone: referral to an obstetrician for further diagnostic examination and prescription of therapy was proposed. Therapy consisted of a three-week course of spiramycin (Rovamycin®) and sulfadiazine (each 3g/day in three doses), repeated once after a two-week interval. Afterwards, a letter giving the test results and recommended therapy and forms to record clinical findings were sent to the patient's midwife, general practitioner and/or obstetrician to whom the patient was referred.

3.3 METHODS

SCREENING

The serum samples were screened in the TIP laboratory for specific antibodies with an ELISA.[183,184] One batch of lyophilised Toxoplasma antigen was used throughout the study period; the same applies for the positive and negative reference sera. Every test included three different positive and three different negative reference sera. The conjugates were obtained commercially (Pasteur anti-human IgG heavy chain, labelled with peroxidase, for the IgG test; Dako anti-human IgM μ chain, labelled with peroxidase, for the IgM test); optimal working dilutions were determined in preliminary tests.
The cut-off value for seropositivity/seronegativity was calculated from the extinction values found in that same test for the three negative reference sera: mean value plus three times the standard deviation for the IgG test as well as the IgM test.

DIAGNOSIS

The two consecutive samples offered to the reference laboratory, in the event of suspected seroconversion, were tested with an ELISA pentatest for IgG, IgM, circulating antigen and circulating immunocomplexes of this antigen bound to IgG and/or IgM.[122,184,185] Diagnosis of a primary infection was based on a change from a seronegative to seropositive result of the IgG test. The results for the other items of the pentatest were not decisive but supported the diagnosis.
The procedures for handling blood samples and data are described in the addendum.

3.4 MATERIALS

Participation in the study and the supply of samples are described below.

3.4.1 PARTICIPATION OF PROFESSIONAL GROUPS

The group of midwives was very positive about the request to participate in the TIP trial; the response of the physicians was hesitant at first.
Ultimately, after a second letter from the Chief Medical Officer of Health, 49% of 1358 general practitioners, 75% of 231 midwives and 75% of 176

obstetricians were willing to participate. There was no response from 29% of general practitioners, 11% of midwives and 17% of obstetricians (table 3.2). It could be calculated from the database that finally 41% of general practitioners, 76% of midwives and 68% of obstetricians really contributed to the study. Table 3.3 compares the size of the different professional groups with the number of women they enrolled in the study, total number of samples taken and the mean number of samples per woman in their practice.

Table 3.2 Participation of professional groups in the TIP study: number and procentual distribution of the different professional groups approached by the Chief Medical Officer of Health, distribution according to their response to the request, and number that finally participated.

	total		positive		negative		no reaction		realized participation	
	n	%**	n	%*	n	%*	n	%*	n	%
general practitioners	1,358	77	670	49	297	22	391	29	557	41
midwives	231	13	173	75	33	14	25	11	175	76
obstetricians	176	10	132	75	15	8	29	17	120	68
total	1,765	100	975	55	345	20	445	25	852	48

* percentage within the professional group

** percentage of the total number of health workers approached

Absolutely as well as relatively the bulk of both subjects and samples was provided by the midwives. As a matter of fact the three professional groups are responsible for an unequal and different part of prenatal care. In the Netherlands in 1987 nearly 56% of deliveries took place under supervision of a doctor, nearly 44% under supervision of a midwife (CBS, Central Bureau of Statistics[186]). In the TIP study this was quite different: 39% versus 61%.

Nearly half of the women who entered the study had a serological proof of prior infection and therefore were tested only once; if they were excluded the mean value of the number of samples per woman (table 3.3) would have been higher and more appropriate to show the true frequency of repeat tests.

Table 3.3 Contribution of different professional groups as indicated by their number, the number of women they enrolled in the study, total number of samples taken and the mean number of samples per woman.

	professional group		
	general practitioners	midwives	obstetricians
n=	557	175	120
number of women	6,568	18,594	5,100
women per GP/M/O: median	7	80	11
mean	12	106	42
number of samples	11,951	42,723	9,012
samples per GP/M/O: median	9	182	17
mean	21	244	75
samples per woman: median	1.3	2.3	1.6
mean	1.8	2.3	1.8

Figure 3.1 shows the distribution of the mean number of samples per woman per GP/M/O. It must be emphasized that this is a mean for each GP/M/O.
Especially for general practitioners and obstetricians the class with a mean of 1-1.2 samples per woman was large: 47.4% of the total of 557 GP's and 29.0% of the total of 120 obstetricians in contrast to 10.3% of the total of 175 midwives. 77% (204) of the 264 GP's in this class, 89% (16) of the 18 midwives and 25% (9) of the 35 obstetricians had a value of 1.0. A mean value of 1 sample per woman may represent a GP/M/O who only enrolled one woman in the study and only took one sample from her (1/1) or a GP/M/O who enrolled several women each of whom was sampled only once (for example, 4/4 or 10/10). Further inspection of the data revealed that it was very common that a sample was taken only once (which means that only one of their patients participated in the study), in

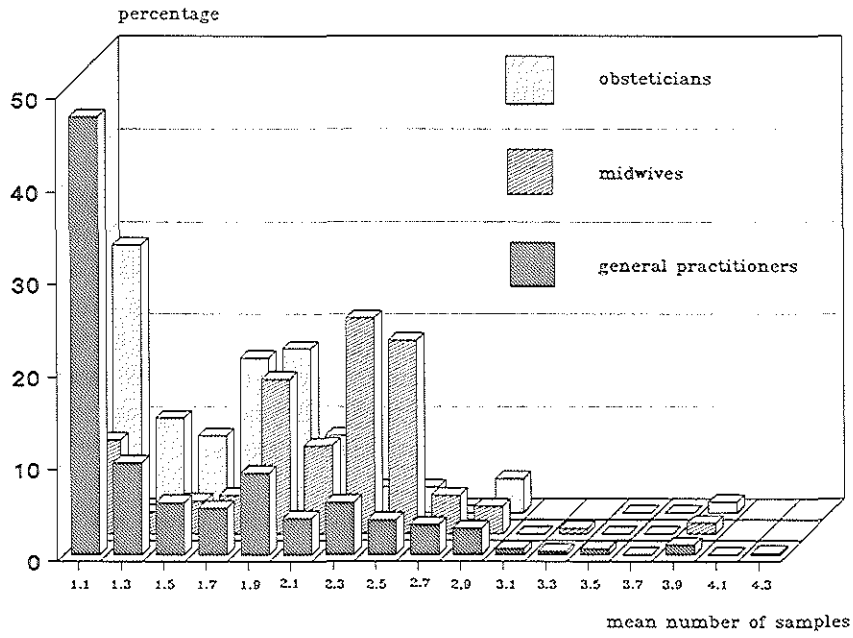


Figure 3.1 Percentage of general practitioners, midwives and obstetricians according to the mean number of samples taken per woman

contrast to a minority who took one sample of several patients resulting in a ratio of e.g. 4/4 or 10/10: among the 204 general practitioners with the value 1.0 108 sent in one sample from one person versus 13 of the 16 midwives and 12 of the 26 obstetricians. This is a striking fact: there were many general practitioners who did not really participate in the study but only took one blood sample from a patient, probably under special circumstances. It may be that in some cases the general practitioner has been asked to take a sample from a woman (who had already been discharged by her midwife) for a confirmatory test after birth of her child. Ratios such as 4/4 or 10/10 indicating that only one sample was taken from each woman can be explained by the fact that some women were seropositive at first examination, some women withdrew after one examination (lack of motivation, spontaneous abortion) and/or by referral of some women to midwives or obstetricians for further prenatal control. The latter was indeed common.

Differences in socioeconomic circumstances of the women may have contributed to the differences between the professional groups.

3.4.2 PARTICIPATION OF ELIGIBLE WOMEN

Women were eligible for the study if they hoped to become pregnant or were pregnant for no more than 20 weeks. In total 27967 women were enrolled in the study. The number of births in 1987 in the province of South Holland was 41,531 which reflects the number of eligible women. Table 3.4 lists the participating women according to age at entry into the study; it also shows whether they were not yet pregnant at that time (nongravida) or were pregnant for the first time (primigravida) or for at least the second time (multigravida). Comparison with the age distribution of mothers of all children born alive in 1987 (Central Bureau of Statistics, CBS) indicates that relatively more young women (under 19 years) participated; the break-even point occured in the 25-29 year age group: relatively fewer women over the age of 30 participated in the study.

Table 3.4 Number of women enrolled in the TIP study according to age and number of the current pregnancy (nongravida = 0, primigravida = 1, multigravida ≥ 2) and the age distribution of mothers of children born alive in 1987 in the Netherlands (CBS).

	number of current pregnancy			total		CBS '87
age (year)	0	1	≥ 2	n	%	%
< 15	0	3	0	3	2.7	1.1
15-19	3	579	175	757		
20-24	27	3,561	2,250	5,838	20.9	14.3
25-29	74	5,191	7,063	12,328	44.0	43.2
30-34	38	1,843	5,336	7,217	25.8	31.8
35-39	9	274	1,365	1,648	5.9	8.3
40-44	2	16	143	161	0.6	1.2
≥ 45	1	2	12	15	0.1	0.1
total	154	11,469	16,344	27,967	100.0	100.0

Figure 3.2 shows the number of blood samples per date of sampling, the number of women per date of entry into the study and the number of children per date of birth according to month and year of the study period.

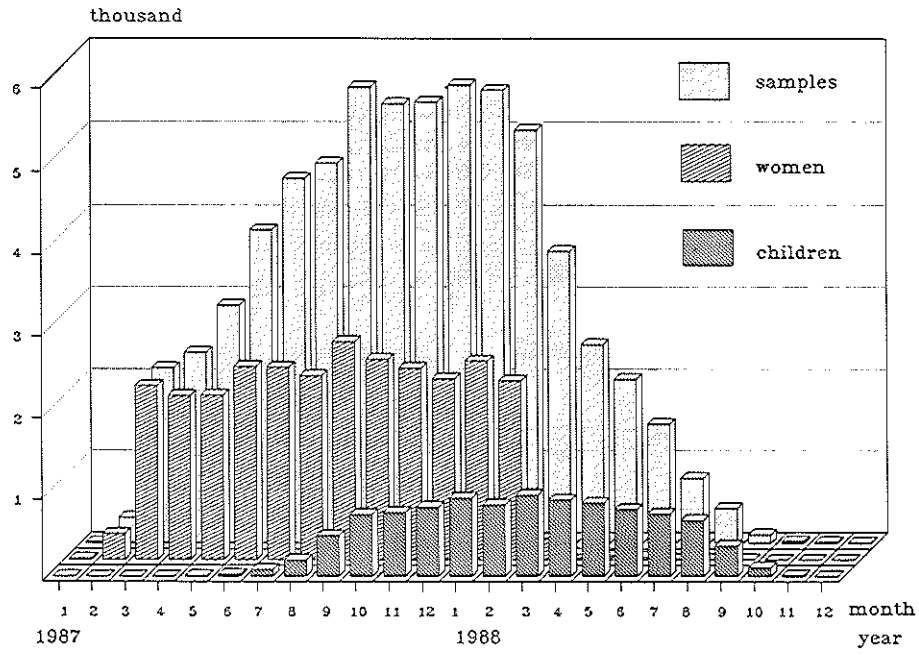


Figure 3.2 Number of blood samples per date of sampling, number of women per date of entry into the study and number of children per date of birth according to month and year of the study period.

Although the study was supposed to begin on the first of March 1987, some people reacted so enthusiastically to the letter from the Chief Medical Officer of Health that from that time on they sent in blood samples. From March 1987 to March 1988 the number remained quite stable. The number of samples exhibited a gradual increase. Two months after the start of the study repeat samples from seronegative women started to arrive. The study was stopped on the first of March 1988; those who were already enrolled were monitored until the end of pregnancy; therefore the number of samples decreased from that time onwards.

3.4.3 BLOOD SAMPLES

Table 3.5 lists the samples of women and children according to serial number

and professional group; "others" were usually paediatricians. This table shows that the contribution of general practitioners decreased after the first sample; this is in accordance with the fact that -after the first visit- prenatal care is often turned over to midwives, whose contribution indeed increased.

Table 3.5 Number of blood samples from mothers (M serial number 1 to ≥ 5) and children (C serial number 1 to ≥ 2) according to professional group (counted on 03-09-1988).

sample	general practitioners		midwives		obstetricians		others		total
serial nr	n	%	n	%	n	%	n	%	n
M 1	6,479	23	17,026	61	4,534	16	5	–	28,044
2	2,226	16	9,896	70	1,937	14	3	–	14,062
3	1,743	14	9,025	73	1,436	12	1	–	12,205
4	1,142	15	5,551	74	825	10	4	–	7,522
≥5	310	18	1,141	67	243	15	5	–	1,699
C 1	945	10	7,372	77	1,300	13	3	–	9,620
≥2	6	13	7	13	12	20	13	31	38
total	12,851	17	50,018	68	10,287	14	34	–	73,190

Figure 3.3 shows the samples with serial number 1 versus the week of gestation for the gestation period 5 to 27 weeks. The mean for this group occured at 12 weeks, the 10-percentile at 7 weeks and the 90-percentile 18 weeks. The directive was not to include women pregnant for more than 20 weeks. We did, however, receive a striking number of blood samples from women in a more advanced stage of pregnancy; these women were tested only once. In addition blood samples from 154 women who wanted to become pregnant were also sent in.

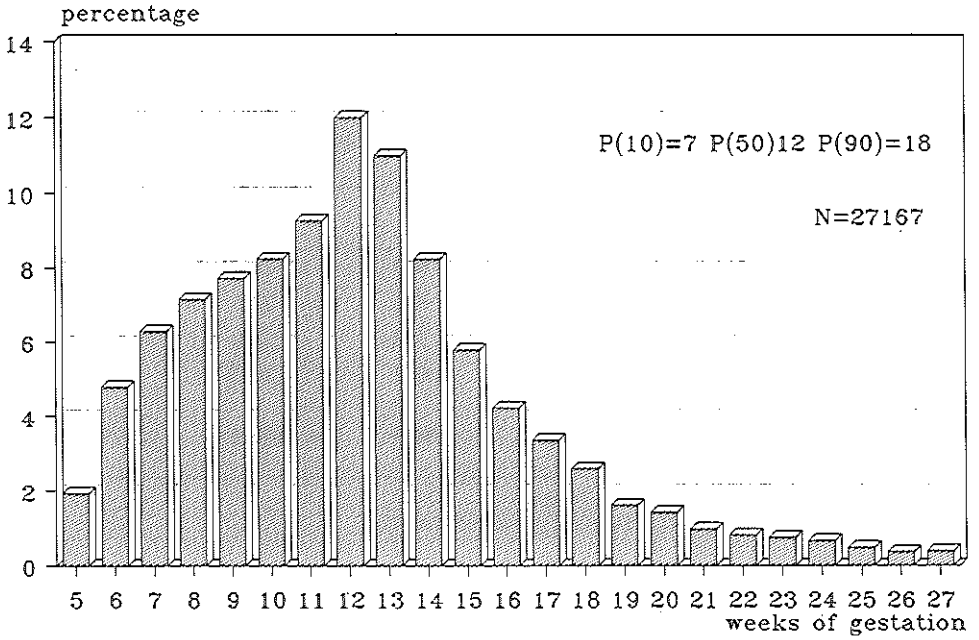


Figure 3.3 Duration of pregnancy (weeks) at the moment of the first blood sampling

3.4.3.1 REPEATED EXAMINATION, REMINDER NOTICE AND DROPOUT

In this section the flow of repeat samples will be described. After the first examination, 15,335 (reference date 01-09-1988) women were advised to continue serological control during pregnancy according to the schedule given in table 3.1. The target date for each particular woman was always stated in the letter reporting the last test result. If no sample had been received one month after the target date, a reminder was sent automatically, in the hope of reducing the dropout rate. This possibility was not available at the beginning of the study which is why the total number of dropouts does not compare with the numbers that can be derived from other tables, e.g. table 3.5. Attempts to reduce the dropout rate were not very successful, as can be seen in table 3.6 which gives the frequency of reminders and the subsequent response.

Table 3.6 Number of reminder notices and the number of subsequently received samples, according to serial number of the sample.

serial number	a number of reminder notices	b number of subsequently rec. samples	a-b dropouts n	a-b dropouts %*	b/a response to reminder(%)**
1	2713	1840	873	28.5	67.8
2	1601	911	690	22.5	56.9
3	1103	213	890	29.0	19.3
4	654	42	612	20.0	6.4
total	6071	3006	3065	100.0	49.5

* relative to the total number of dropouts
** relative to the number of reminders per serial number.

For each woman who had to be screened repeatedly, the prospective study would end at the birth of her child: a blood sample taken from the cord would complete her series.
Ultimately 10,170 of the 15,335 expected samples were received (reference date 1-11-89), i.e. 66%; the missing 34% is an approximation of the overall dropout rate in the course of time; in some cases, however, a sample of maternal blood was collected after parturition instead of a cord sample.

In order to evaluate patient compliance with the proposed schedule, the duration of pregnancy at the moment of sampling can be compared with the recommended schedule (see table 3.1: 18, 24, 32 or 36 weeks). Figures 3.4 a (repeat examination at 18 weeks of gestation), b (24 weeks), c (32 weeks) and d (36 weeks) show the distribution of these groups according to the time the sample was actually taken. The serial numbers are not mentioned in this figure: figure 3.4b, for example, refers to samples with serial number 2 (first sample taken between 14 and 19 weeks of gestation) as well as 3 (second sample taken between 14 and 19 weeks). In all figures the median value coincides with the intended period of gestation. Moreover it is striking that these bimodal distributions exhibit a low second top at a duration of gestation that also coincides with the proposed schedule (for example, in figure 3.4b there is a peak at 24 weeks of gestation and an elevation at 18 weeks).

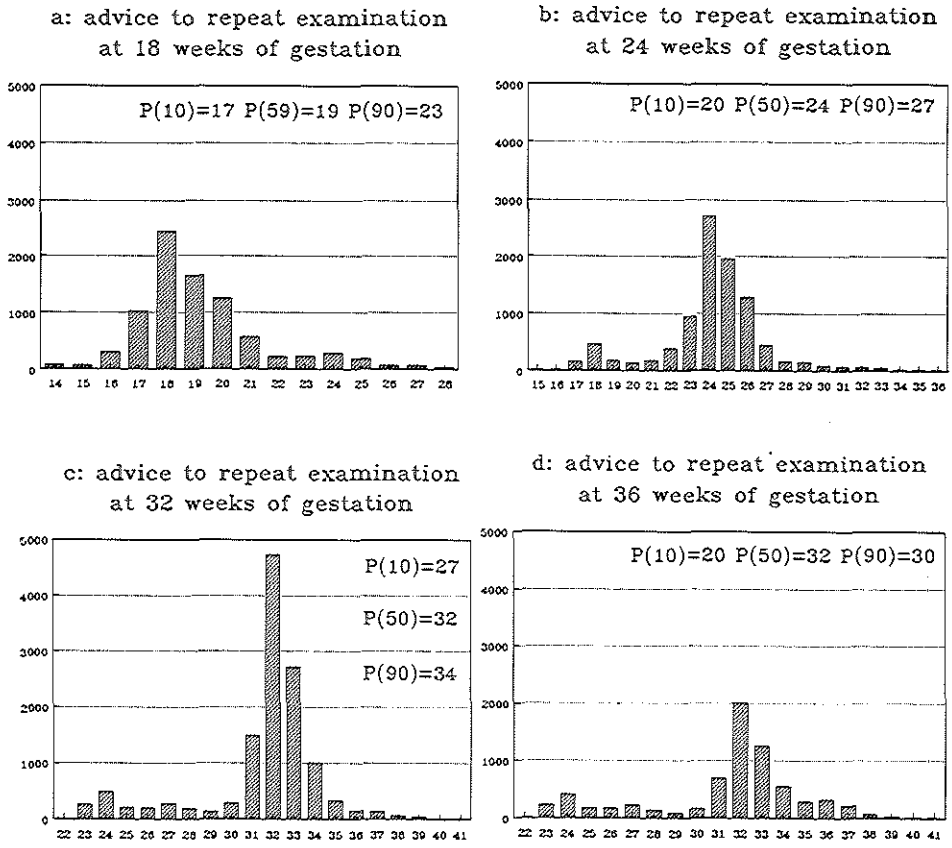


Figure 3.4 Duration of pregnancy (weeks) at the moment of sampling compared with the advised moment for repeat testing

When we set up the schedule for repeat sampling, we tried to realize a mean interval between successive samples of 8 weeks. Between the first and second samples the median interval was 8 weeks (80% confidence interval: 5-13), between the second and third samples 8 weeks (5-12), between the third and fourth samples 7 weeks (5-9) and between the fourth and fifth samples 5 weeks (4-7). Since there was considerable dispersion the 80% instead of the usual 95% confidence intervals are given.
When interpreting the data on the flow of samples, we have to bear in

mind that sometimes a sample was taken from the mother at birth or shortly thereafter instead of a cord sample – especially when the neonate sample was forgotten. These postpartum maternal samples can be found in the group with serial number 5. Secondly, the recommendation to take a sample at 36 weeks often was not followed. Indeed, the schedule was rather strict (36 weeks when the last sample was taken at 30 or 31 weeks), and it was frequently decided to omit the sample at 36 weeks and await birth.

3.5 DISCUSSION

The target population of pregnant women had to be recruited through midwives and physicians who perform prenatal care. Their participation in this study, which signified a considerable workload, was high: midwives 76%, general practitioners 41% and obstetricians 68%. Together they enrolled more than 28,000 women in the study. In total nearly 65,000 blood samples were collected and over 10,000 neonatal samples were taken. From a comparison of the age distribution of participating women with data of the CBS, it can be concluded that the TIP population is representative of the Dutch population of pregnant women in this respect. Although this cannot be supported by data from the present study, the TIP population is also thought to be representative with respect to urbanization since there are rural as well as urban areas in the province of South Holland.

One of the largest problems of prospective investigations is the dropout rate. We tried to keep the number of dropouts low: the result of every test of each woman was sent to her GP/M/O together with the exact date when a new blood sample from that particular woman was expected. If the repeat sample was not received one month after the date mentioned, a reminder note was sent automatically together with a new, nearly completed data sheet (only the actual date of blood sampling had to be filled in). Unfortunately there was not a standard reminder that could be sent when a sample (cord blood or postpartum maternal blood) was not received after the expected birth of the child. Ultimately there was a dropout rate of about 30% during the course of gestation.
The goal of 8 weeks between consecutive blood samples was reached. The median number of samples per woman, as presented in table 3.3 and figure 3.1, is strongly biased since women who were seropositive at first examination and thus were not followed are included in this report.

Compliance with the blood sampling schedule was satisfactory: the median value of gestation at the moment of blood sampling equalled the advised duration of gestation; however there was considerable dispersion around the median value (see figures 3.3 and 3.4).
Even though it was recommended that the first blood sample be obtained as soon as possible after conception or even before, 50% of women were tested for the first time when they were more than twelve weeks pregnant. Thus three months of pregnancy may have passed before the women were informed about the necessary preventive measures.

Participation in the study by physicians and midwives, and their patients, was quite satisfying and resulted in the acquisition of an enormous amount of data. Early in 1988 it was decided that there was no reason to depart from the time schedule and thus the study was stopped on the first of March 1988. Serological follow-up of all women already in the study was continued until the end of their pregnancy. The National Steering Committee, that monitored the study, recommended at that time that serological screening be discontinued in anticipation of the final analysis of the results of the TIP study although primary prevention in the form of health education should be continued. The investigators prepared a new version of the information leaflet for pregnant women that was restricted to primary preventive measures (see appendix 1b).

4 PRIMARY TOXOPLASMA INFECTIONS DURING PREGNANCY

4.1 INTRODUCTION

During the TIP study primary as well as secondary preventive measures were applied in order to reduce the risk of congenital toxoplasma infection. Primary prevention is aimed at pregnant women: reducing the risk of primary infection during pregnancy by health education. To assess how many infections occurred in spite of this, regular serological control was performed. The design, materials and methods of the preventive study have been described in chapter 3, the results of screening will be considered in this chapter.

4.2 RESULTS OF SEROLOGICAL SCREENING

The purpose of the assessment of specific antibodies is dual.
First, it provides an assessment of the seroprevalence from which the magnitude of the population at risk can be estimated. Secondly, it traces seroconversions, which mean primary infection and thus immediate therapy.

4.2.1 SEROPREVALENCE

At first examination 12,797 of the 27,967 women (45.8%) showed specific antibodies. Data on this seroprevalence, stratified according to age and the number of the current pregnancy, are given in table 4.1 and figure 4.1. The remaining 15,170 (54.2%) women who lacked antibodies were considered to be at risk for a primary toxoplasma infection.

Table 4.1 Seroprevalence (IgG/ELISA) according to age for non-pregnant women, for primigravidae and for multigravidae.

	non-pregnant			primigravidae			multigravidae			total		
age	+*	n**	%	+*	n**	%	+*	n**	%	+*	n**	%
≥15	0	0	0	1	3	33	0	0	0	1	3	33.3
15-19	1	3	33	216	579	37	72	175	41	289	757	38.2
20-24	7	27	26	1,428	3,561	40	987	2,250	44	2,422	5,838	41.5
25-29	32	74	43	2,237	5,191	43	3,168	7,063	45	5,437	12,328	44.1
30-34	25	38	66	881	1.843	48	2,682	5,336	50	3,588	7,217	49.7
35-39	6	9	67	154	274	56	781	1,365	57	941	1,648	57.1
40-44	2	2	100	9	16	56	95	143	66	106	161	65.8
≥45	1	1	100	1	2	50	11	12	92	13	15	86.7
total	74	154	54.4	4,927	11,469	45.4	7,796	16,344	49.4	12,797	27,967	45.8

* number of women with specific antibodies

** number of women examined

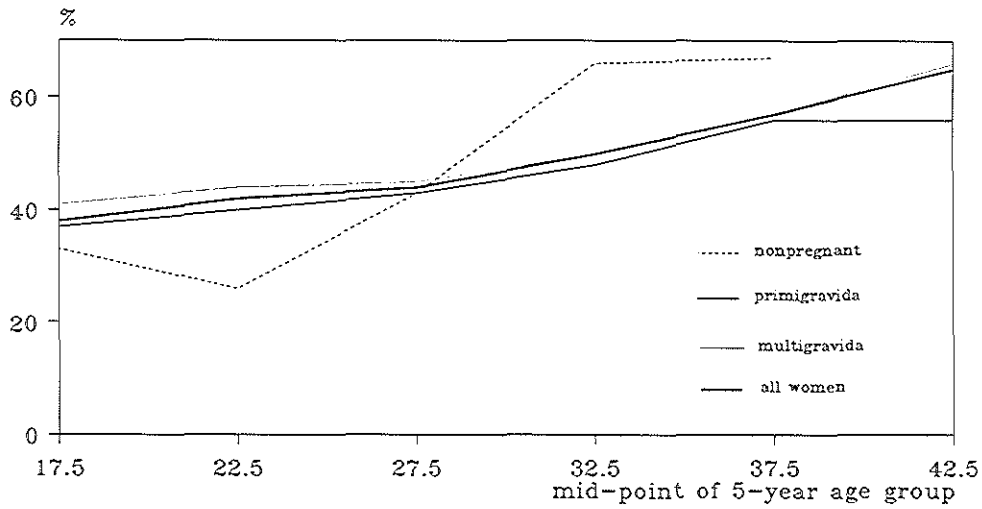


Figure 4.1 Seroprevalence (%) according to age for nonpregnant, primigravida, multigravida and for all participating women

4.2.2 SEROCONVERSION

The appearance of specific antibodies in the serum of previously seronegative women signalizes a possible intercurrent infection. This can be revealed by a positive test for specific IgG in a repeat sample taken during pregnancy, or for specific IgG or specific IgM in a cord sample, or a sample taken from the mother after parturition to complete her series of samples. Suspected infection was reported 244 times. Figure 4.2 gives the findings of further serological evaluation after seroconversion was indicated by the screening test. In 77 cases among 15.170 seronegative women (0.5%) seroconversion was confirmed when the sample was subsequently tested simultaneously with the previous one; thus the criterion for the diagnosis of a primary infection was met. In the other cases the additional diagnostic tests on paired samples showed that there was no real seroconversion; often the quantity of antibodies was marginal and not significantly different from that in the previous sample.

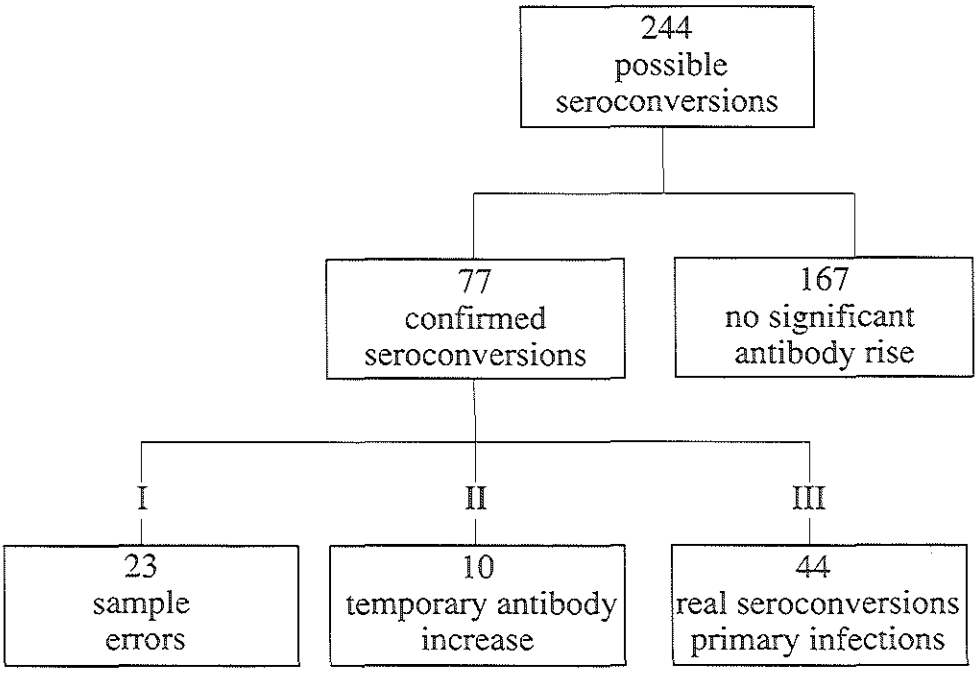


Figure 4.2 Results of serodiagnostic evaluation after demonstration of seropositivity in the screening test

Continued serological follow-up of the 77 women led us to revise the diagnosis 33 times for the following reasons:
In 23 cases (category I in figure 4.2) sample exchange had occurred: the seropositive sample that suggested seroconversion did not belong to the woman concerned. This became clear when a control sample taken after confirmed seroconversion but before therapy was started gave a seronegative result.
In 10 cases (category II in figure 4.2) the IgG antibodies did not persist after seroconversion: repeated serological examination gave a positive test result several times, but specific antibodies could no longer be demonstrated by the ELISA several months later.
Finally diagnosis of an acquired infection was established 44 times (category III in figure 4.2), i.e. 44 of the 15.170 initially seronegative women or 0.3%. The age distribution of these 44 women is given in table 4.2, together with the age distribution of all seronegative women in the study. For 18 of the 44 women this was the first pregnancy, for 16 the second, for 4 the third, for 5 the fourth and for 1 the eighth.
Figure 4.3 presents the 44 primary infections according to the duration of

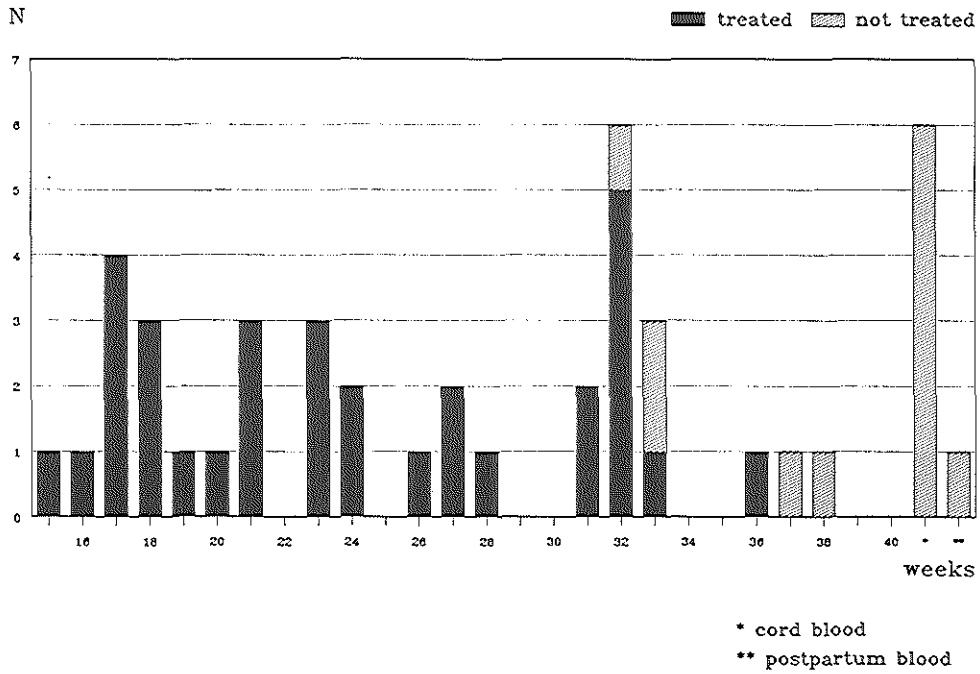


Figure 4.3 Primary infections during pregnancy according to duration of gestation (weeks)

pregnancy at the time that seroconversion was recognized. The intention was to administer pharmacotherapy as soon as possible. Whether this actually occurred is indicated in the figure. The mean duration of observation per woman was 6 months.
No symptoms, suggestive of a primary toxoplasma infection, were reported by the 44 women with a primary infection.

Table 4.2 Occurrence of primary toxoplasma with respect to the number of women at risk (seronegative) according to age group.

age group (years)	number seronegative	primary infection	
		number	percentage
15-24	3,884	10	0.26
25-29	6,891	17	0.25
30-34	3,629	12	0.33
35-44	762	5	0.66
total	15,166	44	0.29

4.3 CASE HISTORIES

In this section some cases from categories I, II and III (figure 4.2) will be presented; the Roman numeral of the case number refers to the category.

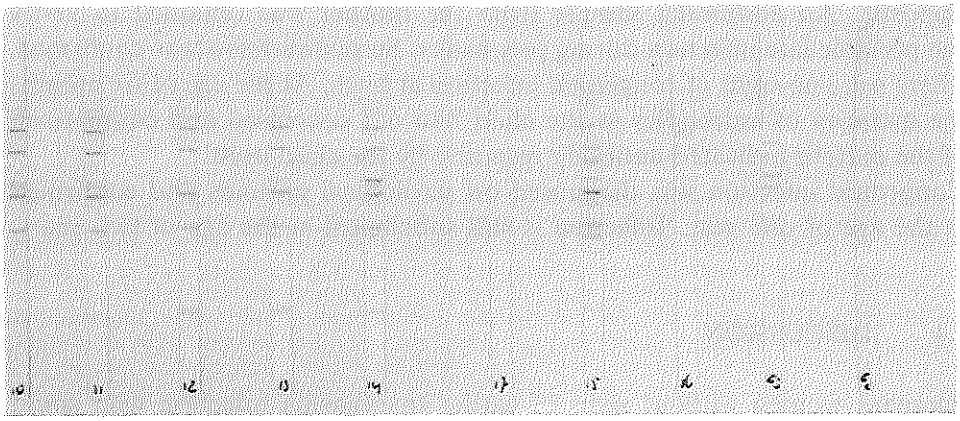
Patient I_1 was seronegative in the 14th week of gestation and seropositive in the 23rd week. Seroconversion was assessed in a simultaneous test of both samples. Quantitative testing of the two samples in the laboratory of the hospital where the woman was under medical supervision also pointed to seroconversion: first sample IgG <1:20, IgM <1:20, second sample IgG 1:80, IgM <1:20. A sample taken twelve days later was found to be negative by both the RIVM laboratory and the hospital laboratory. No more serum was available to compare the samples by immunoblotting. After birth the child as well as the mother was tested serologically: the ELISA, the IIF (<1:16) and the immunoblot were all negative. Sample exchange seemed to be the most likely explanation.

Patient I_2 was seronegative in the 18th week of gestation and positive in the 26th week. Seroconversion was assessed in simultaneous tests. In samples taken 3 and 5 weeks later no antibodies were detected. The titre of the first sample was <1:16 according to the IIF, 1:128 for the second and <1:16 for all subsequent samples. Immunoblotting of all samples showed that the "seroconversion" sample was the only seropositive one and there was no indication of the presence of antibodies in the other samples. It was therefore concluded that the positive sample did not fit in the series and that most probably sample exchange had taken place. Serological testing was continued and she remained seronegative throughout pregnancy.

Patient II_1 was repeatedly seronegative during pregnancy; in a sample taken at 36 weeks of gestation seroconversion was diagnosed. When all samples taken from the woman from the beginning of pregnancy were tested simultaneously it was seen that the extinction values obtained with ELISA had increased after 31 weeks of gestation, although the quantity of antibodies remained below the cut-off value until 36 weeks. The immunoblot revealed an extra line at the level of the 6KD protein that appeared at 25 weeks of gestation. One month after seroconversion, a child was born. Both mother and child were then seronegative according to the ELISA, but the immunoblot continued to show antibodies; at the last examination, 20 months after the birth of the child, the immunoblot had become negative for the child but remained positive for the mother. The results of simultaneous immunoblotting are presented in figure 4.4. It was shown by immunoblotting that antibodies were already present in the four samples (no. 10-13) preceding the seroconversion sample (no. 14).
This case supports the hypothesis that "seroconversion" as a sign of primary infection can be simulated in cases of latent infection.

Serological screening of patient II_2 required special attention.
Seroconversion was assumed from the results of the second screening but could not be confirmed by the diagnostic test. She was further followed and repeatedly screened as proposed. The third and fourth samples were again seronegative, but IgG antibodies were present in the cord sample. At that time all available samples were tested simultaneously by ELISA: antibodies were detectable in all samples, by far the largest quantity being found in the cord sample. The immunoblot was constantly positive. Later serological testing of the mother gave alternating seropositive and seronegative results. Two and a half years after birth of the child, the mother

still had a low quantity of antibodies. Antibodies were never detected in serum from the child by ELISA, although they could be identified on the immunoblot. It is concluded from the persistence of the antibodies, as seen on the immunoblot, that the mother actually had a latent infection.



10	11	12	13	14	17	15	16	63	62
M	M	M	M	M	C	M	C	M	C
					6 mnths		11 mnths		20 mnths

no.	sample date	gestation (weeks)	remarks
10	23-07-1987	12^{+}	
11	10-09-1987	19^{+}	
12	18-10-1987	25	appearance of 6KD line
13	03-12-1987	31^{+}	
14	07-01-1988	36^{+}	seroconversion in ELISA
	09-02-1988	41^{+}	samples taken at birth; no material left for later simultaneous tests

no.	sample date	age of the child	remarks
17	26-08-1988	6 months	
15	16-01-1989		
16	16-01-1989	11	
63	13-10-1989		tested simultaneous with no. 62
62	13-10-1989	20	tested simultaneous with no. 63

Figure 4.4 Immunoblotting of blood samples of mother (M) and child (C). See text: case history II_1.

Patient III_1 was screened serologically three times during pregnancy and was seronegative. A cord sample was tested when her child was born: marginal amounts of IgG and IgM were found, IgG extinction being below the cut-off value, IgM just above; the results of the diagnostic test were just the opposite. An additional sample from the mother was obtained to verify the possible late infection during pregnancy. Seroconversion was confirmed. There were no signs of congenital infection at birth. At the age of six months the quantity of antibodies in the child had not decreased and comparison of the pattern indicated antibody response by the child itself.
Congenital infection was diagnosed on serological grounds and confirmed at the age of one year. Infection is still subclinical at the age of two years.

4.4 DISCUSSION

Pregnant women without signs of earlier toxoplasma infection who could benefit from a preventive programme formed the target population of the study. The criterion was the presence or absence of specific antibodies: those who were seronegative took part in the follow-up screening programme, those who were seropositive did not. Apart from an old, latent infection seropositivity can originate from a very recent infection; but this would be a very rare case among the large number of previously infected women; such a case was not identified in this study. It must be stressed that it is impossible to diagnose recent infection by examination of one serum sample only. There is ample evidence that neither the quantity of antibodies nor the concomitant presence of IgM antibodies is a reliable parameter for distinguishing recent from latent infections (see chapter 7), so we consciously refrained from doing so.

Nevertheless, we were consulted twice because a recent infection was suspected in two women with a seropositive test at first examination (at 9 and 13 weeks of gestation respectively). In one case primary infection was suspected because of lymphadenopathy. From the history, however, it seemed likely that the infection had occurred earlier: during the half year before she became pregnant, the woman had consulted her general practitioner several times because of malaise which prohibited her from fulfilling her job. The woman and later her child were examined following the TIP protocol and at the end of the first year of life congenital infection could be excluded. In the other case infection was suspected because the woman

was tested again in another laboratory and a rise in antibody titre was found.
This woman and later her child were also followed up to the end of the first year and congenital infection was excluded. We have not (yet) been informed about a recognized case of congenital toxoplasmosis among the children of women found to be seropositive at the first examination.
The seroprevalence, as measured in nearly 28,000 pregnant women, increased from 38.2% for 15-19 year old women to 65.8% for the 40-44 year age group. The average seroprevalence was 45.8%. This means that about half of the pregnant women in the Netherlands are at risk for toxoplasma infection. Further analysis of the age-specific seroprevalence data in order to estimate the force of infection will be described in chapter 6.
An initial alert for suspected primary infection was not uncommon; however definite proof was found in only a limited number of cases. The parameter for infection was the change from seronegativity to seropositivity in a simultaneous test of two consecutive samples. No additional quantitative criterion was chosen. In view of the need for immediate treatment, it was considered unacceptable to wait for a significant rise in titre. Of the 77 confirmed seroconversions only 44 were due to primary infections.
Unfortunately 23 cases could be attributed to sampling errors. Once a sample with incorrect personal details (those of a sister-in-law of the woman concerned) was offered, once exchange occurred in the hospital where the sample was taken, twice we discovered an exchange in the TIP laboratory where the procedure was subsequently improved. It was not always possible to trace the origin of exchange; it may have happened at any point in the routing from prenatal clinic to the laboratory. It is important to report these facts, especially since their frequency may outweigh the frequency of the events that we are looking for. It seems realistic to suppose that there were even more sampling exchanges. Every exchange has a counter-part. A certain percentage of errors must always be expected; we tried to reduce this risk by extensive automation of the procedures in our laboratory (see addendum). In relation to the number of samples examined, over 70,000, the number of errors recognized was extremely low ($<0.1\%$).
In 10 cases, one or more samples in addition to the seroconversion sample were seropositive at monthly follow-up, but the women became seronegative again. Sample exchange could not be responsible since several samples taken over a period of more than one month had been seropositive. Moreover this could be proven later on with the immunoblot technique, which provides qualitative information on antibodies that recognize certain Toxoplasma

antigens. Specific antibodies were still measurable with the immunoblot test and the pattern matched with previous ELISA seropositive samples of that particular woman (patients II_1, II_2).
It is hypothesized that the transitory antibody response was the result of exacerbation of an old latent infection. When a toxoplasma infection becomes latent, antibodies persist – it is generally believed – throughout life. This is explained by the endogenous boosting of immunity that occurs when parasites escape from tissue cysts due to transient immunosuppression, which usually goes unnoticed. Theoretically it can be argued that antibodies do not always persist and that some individuals may become seronegative again: sero-reversion.[63] Reactivation of the latent infection in this instance results in an increase in antibody level from below to above detection level, thus simulating seroconversion; this may wrongly be interpreted as a sign of primary infection. Such an event probably does not involve the same risk as a primary infection. Treating such an exacerbation within the framework of our study might result in an overestimation of the benefit of therapy for the prevention of congenital infection. The mechanism described might be activated by the immunosuppressive effect of pregnancy;[187] in that case it is also conceivable that seroprevalence is dependent on the number of pregnancies experienced: women who have undergone seroreversion might become seropositive again as a result of pregnancy. In our study, however, age-specific seroprevalence for women who had experienced several pregnancies did not differ from that for women who were pregnant for the first time. No comparison can be made with the group of women who were not pregnant at the moment of first examination; this group was small and not homogeneous since it comprised both primiparae and multiparae.
One premise of the Health Council is that the risk of congenital toxoplasmosis is restricted to women who are pregnant for the first time.[102] This is not supported by the findings of our study: the occurrence of primary toxoplasma infections was independent of the number of previous pregnancies.
The 44 primary infections were recognized during a limited period of observation of seronegative women. Most women were tested for the first time in about the 12th week of gestation. Moreover around 30% of the seronegative cohort could not be followed to the end of pregnancy. This could have been due to termination of pregnancy by spontaneous abortion or lack of motivation of the woman and/or her midwife/physician. The letter with a request to inform the study centre about the reason of discontinuation was seldom answered. The mean duration of observation was 6 months. In addition it can be assumed that infections that occurred

at the very end of pregnancy probably had not yet resulted in a detectable level of specific antibodies at the time of delivery.[188] This is supported by the history of case III_1, who had only a marginal level of IgG antibodies at delivery; the ELISA IgG screening test was negative; it was the marginal IgM level just above the cut-off value that gave rise to suspicion of an infection.

There is an indication that women over the age of 35 years run twice the risk of acquiring an infection of younger women (table 4.2). Since seroprevalence is higher among older women and thus a smaller proportion of them is at risk, this suggests a higher force of infection among older women. There is no sound explanation for this: why should older women have a more hazardous life style as far as toxoplasma infection is concerned? The trend of delaying pregnancy beyond the age of thirty years might possibly result in an increased risk for toxoplasmosis. However, if table 4.2 is reorganized, using other limits for the age groups, the difference in the chance of infection between younger and older groups is not significant (see chapter 6).

As shown in figure 4.3 a considerable proportion of the infections occurred in the last trimester. Diagnosis of these late infections, that were not recognized in time to administer therapy before delivery, actually cannot be considered a benefit of the screening programme. Secondary prevention is of little use in reducing the risk of congenital infection due to maternal infection late in pregnancy. Women are strongly motivated to lead a healthy life at the beginning of their pregnancy, but they probably are not convinced sufficiently of the continuing need for preventive measures up to the end of pregnancy. It is still a matter of public opinion that the foetus is only susceptible to infectious or toxic agents during the first months of pregnancy. Therefore stimulation of primary prevention at the end of pregnancy will be much more appropriate and profitable. There is little resistance to primary preventive measures for the duration of pregnancy; but the women were rather distressed when infection developed in spite of their conscious compliance with the recommended measures; none of them remembered accidental exposure to potential infectious sources. On the other hand, the yield of secondary prevention could be increased if the first examination of pregnant women is advanced and more infections occurring early in pregnancy can be traced.

Estimation of the true number of primary infections that can be expected to occur in Dutch pregnant women, based on this prospective study, is discussed in chapter 6.

4.5 CONCLUSIONS

During a prospective investigation of 15,170 initially seronegative women who followed primary preventive measures, only 44 (0.3%) primary toxoplasma infections were detected by repeated serological screening (or 0.16% of the total cohort of 27,967 women regardless of their immune status). Secondary prevention was applied as soon as possible; in more than 25% of cases, however, pregnancy had already come to an end and the moment for effective therapy had already passed when the infection was recognized. This means that the profit of secondary prevention was relatively limited. Moreover more than 50% of the women did not come under medical supervision before 12 weeks of gestation, so the beginning of pregnancy had not been covered sufficiently by the preventive programme: on the one hand some of these women would not have been aware of the necessary preventive measures and on the other hand infections occurring early in pregnancy have been missed.

Probably the low number of infections reflects an effect of primary prevention, but this cannot be proved by the present investigation. It must be kept in mind that primary prevention may have been enforced by the repeated blood sampling, reminding the pregnant woman as well as her midwife/ doctor of the necessity of primary preventive measures.

5 INCIDENCE OF CONGENITAL TOXOPLASMA INFECTIONS

5.1 INTRODUCTION

The TIP study was directed at the prevention of congenital toxoplasma infection. The first possibility was to prevent primary toxoplasma infection in pregnant women. If this was not successful, a second possibility remained: prevention of transmission from the mother to her unborn child by treating her as soon as infection was diagnosed. In chapter 4 the occurrence of acquired infections when primary preventive measures were applied was considered. The infected women, traced by repeated serological examinations, were treated. The results of these secondary preventive measures are discussed in this chapter. Since treatment of a pregnant woman also influences the course of the possible foetal infection,[146] evaluation of the applied secondary preventive measures may not be restricted to assessment of the incidence of congenital infection but should also include information about the course of infection in the child.
Definite assessment of the incidence at birth is barely possible since absence of symptoms at birth or in the first months of life does not exclude infection. Symptoms may remain subclinical even for a decade.[84,86,87]
The diagnosis of a congenital infection can be made or confirmed by demonstration of antibody production by the child itself. The serological tests have to be repeated several times during the first year of life in order to observe the evolution of the child's immunoresponse as the passively acquired antibodies of maternal origin disappear. If the child is infected, it will not become seronegative due to its own antibody production.
Paediatric and ophthalmological examinations are indicated in order to determine possible changes in the expected clinical picture.

In this chapter our observations during the first year of life of the children of infected mothers are described. On the basis of the follow-up findings the incidence of congenital toxoplasma infections is assessed.

5.2 DESIGN AND ORGANIZATION OF THE FOLLOW-UP STUDY

In this follow-up study all children of infected mothers were observed for one year. The study consisted of (neuro)paediatric, ophthalmological and laboratory examinations soon after birth, at the age of six months and around the first birthday.
The RIVM coordinated the study; moreover the serodiagnostic and parasitological tests were performed in the parasitological reference laboratory of the RIVM. Paediatric examination was performed at the hospital of delivery. Ophthalmological examination of all children was performed by one ophthalmologist[b] at the Institute of Ophthalmology of the Erasmus University in Rotterdam. The reason for this was to exclude inter-observer variation because not all ophthalmologists are experienced in examining newborn children. Once a month a special ophthalmological clinic was organized for these children.
As usual, the patient's paediatrician and general practitioner were in charge of follow-up care and counseling. In addition the study coordinator was present at the ophthalmological consultation to explain to the parents the necessity of repeated examinations to be sure whether the child had been infected or not and to give them further information.

5.3 SUBJECTS AND METHODS

During 1987/1988 27,967 women participated in the study in the province of South Holland. Repeated screening for specific antibodies against *Toxoplasma gondii* was recommended for the 15,170 who were initially seronegative. Children whose mother went through an apparent primary infection (see chapter 4) were examined extensively in order to exclude congenital infection.

5.3.1 STUDY POPULATION

The children included in the follow-up study were children potentially at risk for a congenital toxoplasma infection because of infection of the mother during pregnancy.

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[b] Prof. dr. P.T.V.M. de Jong.

A primary toxoplasma infection was suspected in 54 pregnant women (see chapter 4 and figure 4.2). The infection was confirmed later on by seroconversion in 44 women (category III); 12 were not treated because the infection was not recognized before delivery. In one other case treatment was started only one day before delivery. It was concluded retrospectively – after repeated serological control – that ten of the 54 women had actually suffered exacerbation of a latent infection; children of these women were also included in the follow-up study (category II).

5.3.2 PAEDIATRIC EXAMINATION

The paediatric examination comprised a general physical examination with special attention for icterus, exanthema, hepatosplenomegaly and neurological examination. Ultrasound examination of the skull was performed in order to detect ventricular dilatation and intracerebral calcifications.
Blood was tested for haematological and chemical parameters.

5.3.3 OPHTHALMOLOGICAL EXAMINATION

The eyes were dilated with one drop of 5% phenylephrine and 0.5% cyclopentolate. Alignment of the eyes was tested by the corneal light reflex. After administration of one drop of 0.4% oxybuprocaine a lid speculum was inserted. The outer eye and anterior chamber were examined with a hand-held slit lamp. Media opacities were excluded by means of the red fundus reflex; the fundus of each eye was examined by indirect ophthalmoscopy without indentation as far as the equator.

5.3.4 SEROLOGICAL EXAMINATION

Serum samples were tested with an ELISA for specific IgG, IgM, circulating antigen and circulating immunocomplexes with IgG or IgM.[122,184,185] Maternal and neonatal serum samples were always tested simultaneously in order to permit quantitative comparison of the extinction values. The antibody patterns of mother and child were qualitatively compared by immunoblotting, which was performed according to Laemmli and Towbin with minor adaptations.[189,190]

5.3.5 PARASITOLOGICAL EXAMINATION

If a specimen of placental tissue taken after delivery arrived at the laboratory within several hours, a classical mouse isolation test was performed; moreover a direct immunofluorescent assay (DIF) and an ELISA antigen test for Toxoplasma were also performed.

5.3.6 DIAGNOSTIC CRITERIA

The persistence of specific antibodies to *Toxoplasma gondii* beyond the age of one year was decisive for the diagnosis of congenital infection. This was determined by the ELISA.
During the study we also relied on the results of immunoblotting. If more lines were seen on the immunoblot of the child compared to the maternal pattern this was thought to be indicative of the presence of antibodies produced by the child itself. It was assumed that with this method early prediction of a possible congenital infection before the end of the first year was feasible. The final conclusion, however, was always based on the ELISA result.

5.4 RESULTS

Forty-two of the 44 children in group III could be followed up to the age of one year. One child was examined only once in his first month of life before the parents withdrew him from the study on philosophical grounds; there were no abnormal findings at that time but a diagnosis could never be made or excluded. The other child was observed only during the first half year of live but information has been obtained about the outcome in this case. Nine of the ten children in group II could be followed for one year.
One child was only examined shortly after birth; there were no signs of infection at that time.

5.4.1 PAEDIATRIC FINDINGS

Physical signs that could correspond to congenital infection were reported

by the paediatrician in three cases only. The first concerned the child of a woman who was infected during the last trimester of pregnancy, as indicated by the presence of antibodies in cord serum. After 40 weeks of gestation a baby was born by vacuum extraction after rupture of the membranes without sufficient contractions. Because of the ruptured membranes and an established leucocytosis prophylactic antibiotics were administered. The child became icteric and was treated by phototherapy. The child was dismissed from hospital on day 10 and developed well. At the age of five months the child was seronegative according to the ELISA with a slight indication of remaining maternal antibodies on the immunoblot; at the age of 11 months congenital toxoplasma infection was definitely excluded.
The second child underwent surgery for duodenal atresia. Immunoblotting of maternal and cord serum suggested congenital toxoplasma infection. The child was hypotonic and seemed retarded. There were neither skull abnormalities, as indicated by ultrasound and x-ray examination, nor ophthalmologic abnormalities. The child was treated with pyrimethamine and spiramycin. At the age of 8 months the child was seronegative according to ELISA; the immunoblot at that time also indicated disappearance of maternal antibodies without response of the child itself.
Intracerebral calcification was observed in the third child as the only clinical sign of congenital infection; at the age of 5 months the infection was confirmed serologically: an increase in antibodies was seen on a simultaneous immunoblot of two consecutive samples taken at 2 and 5 months, respectively.

5.4.2 OPHTHALMOLOGICAL FINDINGS

Chorioretinitis was diagnosed within the first year of life in two of the 43 examined children (4.7%), both from group III (figure 4.2): III_2 and III_3. In child III_3, described in section 5.5, a lesion with central pigmentation surrounded by a bright zone was observed near the macula of the left eye; the child was then three weeks old. Re-examination at 12 weeks of age showed that pigmentation of the lesion had increased. Child III2 was not examined ophthalmologically until the age of 7 months; there was a temporal, highly pigmented chorioretinitis scar in the left eye.
Duane retraction syndrome was established in one child, who exhibited convergent strabismus with torticollis, limitation of abduction and to a lesser extent adduction of the left eye. Duane syndrome has been described as a manifestation of congenital toxoplasmosis;[191] in this child, however, congenital toxoplasmosis was excluded on the basis of serology.

Some ophthalmological findings were encountered that are not typical of toxoplasmosis:
- intraretinal haemorrhages were seen at funduscopy two weeks after birth in two babies;
- two linear foci of pigmentation in one eye, resembling slight benign hyperplasia of the retinal pigment epithelium;
- several patches of glittering "drusen"-like material in the choreoidea;
- mild iris hyperaemia at four weeks and a tiny depigmented scar at 14 months.

Apart from the latter child with serological evidence of congenital toxoplasma infection none of these children were infected.

5.4.3 SEROLOGICAL FINDINGS

Table 5.1 presents the serological findings for the children of group III; the earliest serological signals suggestive of congenital toxoplasma infection are presented.
Specific IgM antibodies were present in a cord sample in one case only. A discrepancy in the pattern of antibodies on an immunoblot with respect to a pair of blood samples from mother and child taken at birth gave rise to suspicion in 11 cases: in two cases observation of the serological response proved that the children were infected; in nine cases they were not. Quantitative comparison by ELISA extinction of IgG antibodies in maternal and neonatal samples taken soon after birth was not informative.
In six children the level of IgG antibodies at the age of six months was below that of the mother, but the immunoblot was suggestive of antibody production by the child itself. One proved to be infected. In another case, additional assays proved that this was a false-positive reaction due to natural antibodies; this is presented in section 5.5 as case III_7.
For seven children both quantitative comparison of the level of antibodies by ELISA and qualitative comparison by immunoblotting from the age of 6 months pointed to congenital infection; all were infected. One of these seven had had IgM antibodies in a blood sample taken at the age of two months and tested in the hospital laboratory.
For 18 children serology never indicated antibody production by the child whereas the maternal antibodies were gradually disappearing; one of the children was not tested serologically after the age of two months; ophthalmological examination, however, revealed that he was infected (case III_3).

Nine children of group II were followed during their first year. Six of them had one suspicious immunoblot, but they became seronegative within one year of observation so that a congenital infection could be excluded.

Table 5.1 Results of serological examination during the first year of life of 42* children of infected mothers

earliest serological finding suggesting congenital infection	observed in n children	congenitally infected
at birth:		
- presence of specific IgM antibodies	1	1
- suspicious immunoblot and antibody level of child equal to that of the mother	11	2
at the age of 6 months or more:		
- suspicious immunoblot and antibody level of child below that of the mother	6**	1
- suspicious immunoblot and antibody level of child equal to or more than that of the mother	7	7
no serological signs of infection	17	0
total	42	11***

* From 2 children out of the cohort of 44 no serological data at 6 and 12 months were available.

** natural antibodies were demonstrated in the serum of one child; see text

*** One of the two children mentioned above (*) was congenitally infected which was proved by fundoscopy; this child was not included in this list because of the lack of serological data.

5.4.4 PARASITOLOGICAL FINDINGS

Out of group III (n=44) 23 placental samples were available, 10 of which could be inoculated into mice, out of group II (n=10) 3 placental samples were available, 2 of which were tested in mice. None of the samples yielded a positive result in the direct immunofluorescent assay or the ELISA for specific Toxoplasma antigens. Inoculation of placental material into mice

only indicated the presence of parasites once: one of the three inoculated mice died from toxoplasmosis. This is described in section 5.5 as case II_3. At the end of one year follow-up it was concluded that the child was not infected. Only from one of the infected children placental material was sent in; the mouse test was negative.

5.4.5 PROVEN CONGENITAL TOXOPLASMA INFECTIONS

The number of primary infections during pregnancy, subsequent congenital infections and corresponding transmission rates according to time of maternal infection and treatment are presented in table 5.2 and figure 5.1. A diagnosis of a congenital toxoplasma infection was established for 12 of the 43 children for whom the outcome could be assessed. This equals a transmission rate of 28%. One child was only examined at birth, so it is not known whether he is infected. Congenital infection was excluded for all children of mothers of group II.

Table 5.2 Number of primary infections during pregnancy, subsequent congenital infection and corresponding transmission rate, according to time of maternal infection (trimester).

	treated			untreated			total		
	PTI*	CTI**	TR***	PTI*	CTI**	TR***	PTI*	CTI**	TR***
	n	n	%	n	n	%	n	n	%
trimester:									
second	20	2	10	-	-	-	20	2	10
third	12	1	8	11	9	82	23	10	43
total	32	3	9	11	9	82	43	12	28

* PTI Primary toxoplasma infection
** CTI Congenital toxoplasma infection
*** TR Transmission rate

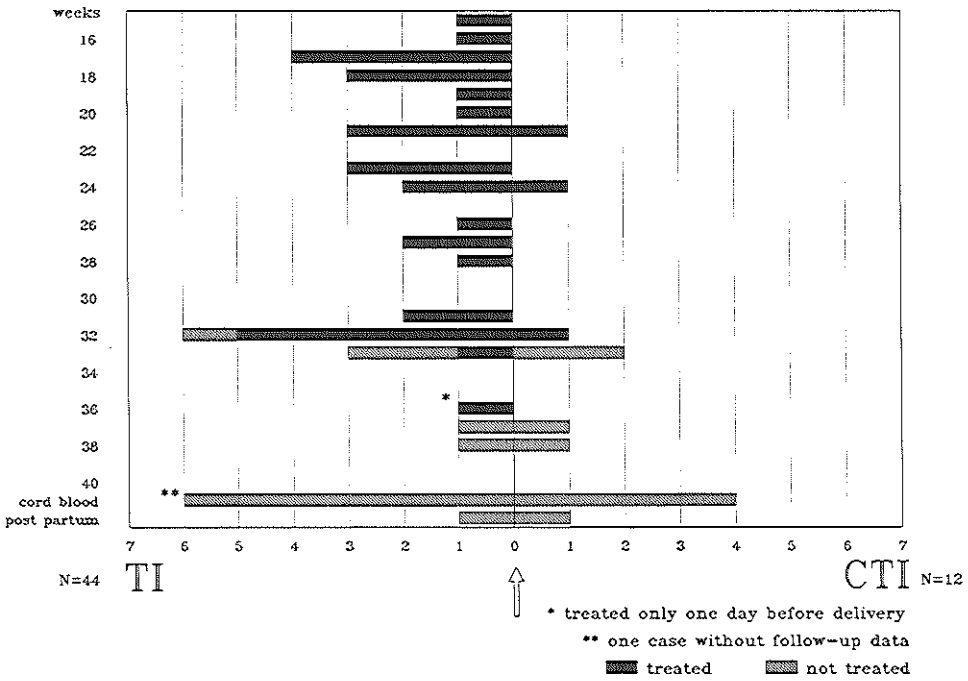

Figure 5.1 Maternal toxoplasma infections (TI) according to duration of pregnancy and subsequent congenital toxoplasma infections (CTI); for treated and untreated mothers.

No serious neurological sequelae of congenital toxoplasma infection were present in the infected children, except intracerebral calcifications in one child. Two children had ocular toxoplasmosis. In nine children formation of specific antibodies in the course of the first year of life was the only evidence of congenital infection.
The infected children are in a continued follow-up study with yearly paediatric, ophthalmological and serological examinations. If relevant the findings beyond the age of one year are mentioned in the case histories.

5.5 CASE HISTORIES

To illustrate the problems encountered during the follow-up examinations to exclude or diagnose a congenital toxoplasma infection several cases are described below. The Roman numeral refers to the categories used in chapter

4 (II exacerbation of a latent infection of the mother during pregnancy, III proven primary infection during pregnancy); the Roman numeral is followed by the serial number which follows the numeration of the cases in section 4.3.

Patient II_3 was seropositive in the 29th week of gestation after she had been seronegative twice during the first 19 weeks of gestation. She was not treated because she lived on a barge and could not be reached. After delivery, maternal and cord samples as well as placental tissue were examined. Surprisingly both blood samples were seronegative according to ELISA, but antibodies were detectable by immunoblotting. Direct immunofluorescent assay of placental tissue revealed no parasites; the antigen test (ELISA) of this sample was also negative; one of the three mice inoculated with homogenized placental tissue, however, became infected. Additional samples were taken throughout the first year of life of the child who developed in a perfectly normal fashion without any (neuro)paediatric or ophthalmological defects. The child was always seronegative, the mother was seropositive every now and then. Additional quantitative assessment with an IIF indicated that the mother had an antibody titre of only 1:16. It is questionable whether the diagnosis of a primary infection was valid.
From these puzzling serological findings it was concluded at last that the mother most probably had a latent infection which had spread into the placenta. The antibody level would have decreased below the level of detection; reactivation of the old infection may have been responsible for the increase in antibodies and the spread into the placenta; the foetus, however, was not reached.

Patient III_2 was tested three times during pregnancy with a negative result up to the 22nd week of gestation. In a sample taken at 33 weeks seroconversion was indicated by the appearance of both IgG and IgM antibodies. She received a three-week course of spiramycin (3 grams daily) and sulfadiazine (3 grams daily). We were not informed about the birth by caesarean section of a dysmature child (1900 gram) after 38 weeks of gestation, nor did we receive samples from the mother and child. On retrospective inquiry it appeared that there were no congenital defects.
Ultrasound and x-ray examination of the skull was not performed. The physician concluded from the results of testing of a sample of the child in the hospital laboratory, i.e. IgG-positive and IgM-negative, that "the child probably had enough maternal antibodies".

At about the age of six months the child finally entered the follow-up study. Serological examination showed that both mother and child were IgG-positive but IgM, circulating antigen and circulating immunocomplexes with IgG or IgM were negative; the quantity of IgG antibodies in the child exceeded that of the mother. Immunoblotting suggested antibody response by the child itself. At ophthalmological examination a highly pigmented chorioretinitis scar was seen temporally in the periphery of the left eye.
It was concluded that the child had congenital toxoplasmosis.

Patient III_3 was seronegative according to repeated serological tests up to the 24th week of gestation. In a sample taken at 32 weeks both IgM and IgG antibodies had appeared which indicated primary infection. Therapy of the mother was started (3 grams of spiramycin daily for 3 weeks and 3 grams of sulfadiazine daily for 2 weeks). The woman, known to have gestational diabetes and hydramnion, gave birth after 38 weeks of gestation to a son weighing 4110 gram by caesarean section because of high complete breach presentation, as we were informed afterwards. There were no abnormalities at paediatric and neurological examination. No intracerebral calcifications were visualized by X-ray examination of the skull. No samples were taken at birth, but blood samples from mother and child taken 11 days postpartum were provided. The mother still had IgG and IgM antibodies, the child only IgG antibodies. Neither quantitative comparison (ELISA) nor qualitative comparison (immunoblotting) indicated antibody response by the child at that time. Funduscopy at two weeks, however, revealed a chorioretinitis lesion near the macula in the left eye, with central pigmentation surrounded by a bright zone. It was concluded that the child was congenitally infected with *Toxoplasma gondii*, although serological confirmation had to be postponed until maternal immunity had waned and the child's own response became measurable. On the one hand the patient's doctor tended to doubt the diagnosis because of the alleged discrepancy between the serological and ophthalmological findings; on the other hand, he felt that repeated serological examination during the first year was not necessary because of the diagnosis based on ophthalmological findings. The doubts of the physician demotivated the parents to continue participation. The child was seen for the last time at the age of 7 months. There was convergent strabismus and funduscopy again revealed the chorioretinitis scar, with even more pigmentation, in the macular region of the left eye.
The parents did not consent to blood sampling and withdrew from the study.

Patient III_4 had no specific antibodies to *Toxoplasma gondii* in the 11th week of gestation. In the next sample, taken at 21 weeks, IgG and IgM antibodies were detectable. Treatment of the acquired infection was started. After a gestation of 41 weeks a girl was born. A cord sample and a sample from the mother taken at delivery were not obtained. Six weeks after birth samples from mother and baby could be tested serologically. The mother had both IgG and IgM antibodies but neither circulating antigens nor immunocomplexes. In the sample of the child only IgG was measured, the level being less than that of the mother. Immunoblotting could not be performed because of lack of material. Although it was initially thought that no further control was indicated because of the absence of specific IgM, we explained that this did not rule out the diagnosis and that continued follow-up was necessary. The child developed well; ultrasound examination of the skull showed normal ventricles and no calcifications in the brain; no ophthalmological signs of toxoplasmosis were seen. Specific IgG antibodies were measurable in a sample taken from the child at the age of six months; the level, however, was low; the mother was positive for both IgG and IgM. From the discrepancy in antibody pattern between mother and child, however, it was assumed that the child itself produced antibodies, which is a sign of congenital infection. When the child was 11 months old, mother and child had equally low quantities of IgG. The immunoblot was not different from the one six months before: again a discrepancy between mother and child was evident. Notwithstanding the supposed congenital infection, the child developed well; there were neither complaints nor paediatric or ocular signs of infection. Surprisingly, antibodies could no longer be detected by either ELISA or immunoblotting in a sample taken when the child was 26 months. The mother had a low level of specific IgG antibodies, detectable with both techniques. According to the study criteria a congenital infection was diagnosed and annual examinations was recommended.

Patient III_5 was seronegative for Toxoplasma throughout pregnancy. In a cord sample specific antibodies were present. A simultaneous test of the last pregnancy sample with the cord sample confirmed seroconversion and proved that a primary infection had occurred after 35 weeks of gestation (the time that the last maternal sample was taken). The healthy newborn girl showed no signs of congenital infection. Ophthalmological examination showed no abnormalities at 2 and 8 months. No blood samples were provided until the child was 11 months old. Serology performed at that time proved congenital infection of the child: the IgG antibody level of the sample from

the child was higher than that of the mother's sample; moreover the immunoblot pattern deviated from that of the mother. Infection was still subclinical: there were no neuro(paediatric) or ophthalmological abnormalities. In addition it can now be reported that the child had a chorioretinitis scar in the right eye at the age of two years.

Patient III_6 was seronegative after 16 weeks of gestation but seroconversion was diagnosed at 24 weeks: both IgG and IgM were present.
Treatment was instituted. Monthly serological tests until delivery showed persistence of IgG and IgM. After 39 weeks of gestation a daughter weighing 3460 grams was born. Placenta examination gave the following results: direct immunofluorescent assay: no parasites observed; antigen test (ELISA): negative; mouse inoculation: negative. In the cord sample IgG antibodies were present but not IgM. The immunoblot suggested antibody production by the child itself, although this finding could not be reproduced two months later. No clinical signs of possible infection were revealed by either ophthalmological examination or ultrasound examination of the skull. At the age of one year, low levels of IgG antibodies were still detectable by both ELISA and immunoblotting. The patterns found for mother and child did not differ. In view of the criterium of antibodies persisting beyond the age of one year a congenital toxoplasma infection was diagnosed. Five months later, however, the child was seronegative according to ELISA. Unfortunately no serum was available for a repeated test. Our request for a new sample was not granted. Ophthalmological examination at one and at two and a half years showed no defects.

Patient III_7 was seronegative three times during pregnancy but in the 32nd week of gestation seroconversion was shown, a primary infection was diagnosed and the mother was treated. At birth antibody levels in the maternal and neonatal samples were equal, without any indication of a different antibody response by the child itself seen on the immunoblot. An antigen test (ELISA) of placental tissue did not reveal Toxoplasma antigen, the direct immunofluorescent assay for parasites in this specimen was unsuccessful. At six months the immunoblot suggested a decrease in maternal immunity; no antibodies (IgG or IgM), circulating antigen or specific IgG immunocomplexes were detected with the ELISA, but IgM immunocomplexes were present. This was indicative of activity of a toxoplasma infection although there was no further evidence at that time. Three months later the results of ELISA were unchanged: no specific antibodies, only a positive

result for IgM immunocomplexes. The child's immunoblot showed a line that was not present on the mother's immunoblot; otherwise maternal antibodies had nearly disappeared.
As time went by the complexes disappeared, and no antibody response was present. It was presumed that the child had "natural antibodies" that disturbed the complex test (ELISA) and the immunoblot. This could be confirmed by indirect immunofluorescent assays of the child's serum which showed polar staining of the parasites, known as the capping phenomenon.

5.6 DISCUSSION

Observation for at least one year is considered necessary in order to assess which children really have a congenital infection.[192] This is illustrated for example by the history of case III_5. Considerable effort was required to motivate parents of apparently healthy babies to remain in the follow-up study. The limited therapeutic perspective combined with the burden of the study, psychologically as well as practically, explain this lack of motivation. Although extensive up-to-date information was provided to the midwives and physicians by the study centre, some parents received inconsistent information – which caused considerable confusion. Follow-up of cases III_2, III_3 and III_4 was nearly terminated due to misinterpretation of the serology by the patient's doctor.
Regular feedback of paediatric findings was a problem. We found that physicians often departed from the proposed protocol if the child was doing well at first examination. Apparently they were not convinced of the need of extensive investigations. At this point, one might question the extent of the protocol. If it is thought that a congenital infection should always be treated during the first year of life, it is not acceptable to delay diagnosis: as soon as infection is recognized treatment should be administered and repeat examinations should be performed until the first year. If it is thought that congenital infection should only be treated in the event of parasitic activity, the protocol should be even more comprehensive (for example, including a spinal puncture and funduscopy). On the other hand, if it is thought that treatment is only indicated in the event of severe manifest lesions, then – except for the investigator's interest- the protocol might be reduced to serodiagnostic tests at the end of the first year of life.
Two of the 44 parents terminated their participation in the follow-up study. For one of these children, however, the outcome could be determined from

the early recognition of a chorioretinitis lesion, although no further blood samples were available to confirm the diagnosis serologically. The ophthalmologist who saw the child later on because of strabismus confirmed the ophthalmological diagnosis of a chorioretinitis. Ultimately the outcome for only one child out of 44 (2%) was lacking.

Quantitative comparison of simultaneously tested samples from mother and child may provide information about antibody production by the child only after maternal antibodies have (partially) disappeared. The evolution of the child's own antibody response due to congenital infection can also be assessed in a simultaneous test of successive samples from the child; the laboratory should freeze all samples to make this possible.[193] In addition to quantitative serology immunoblotting may be a promising qualitative technique for early detection of antibody response in the infected child if the serology is still dominated quantitatively by passive immunity.
Nevertheless this technique, which is still under evaluation, is not yet optimal. In our study, a sign of possible infection on the immunoblot nearly always coincided with a sign of possible infection from ELISA: an antibody level for the child equal to that for the mother, thus indicating antibody response in the child while maternal immunity was decreasing.
In cases III_4 and III_6 antibodies persisted beyond the age of 12 months, but 5 and 14 months later, respectively, these children were seronegative. Their mothers exhibited a markedly decreased antibody level but were still seropositive. Restriction of the criterium for congenital infection from persistence of antibodies to a measurable increase of antibodies in the child's serum should be considered.
IgM antibodies prove foetal infection since they normally do not pass through the placenta and thus must be produced by the child. Our data confirm that IgM detection is not a sensitive parameter since only 1 out of 12 infected children was IgM-positive at birth. Two other samples obtained at birth from a child were IgM-positive; later inquiry, however, revealed that they were actually maternal blood samples.
Parasitological examination of placental tissue did not yield positive results for any of the infected children in our series. On the other hand, there was one positive mouse test, i.e. for patient II_3, indicating reactivation of a latent infection during pregnancy; parasites must have been present in the placenta since one mouse inoculated with the specimen died from toxoplasmosis. Examination of the child throughout the first year of life proved that the child was not congenitally infected; thus parasites were present

in the placenta but did not infect the foetus. These findings are not in accordance with those of French investigations in which the presence of *Toxoplasma gondii* in the placenta was presumed to prove foetal infection; the authors frequently isolated Toxoplasma from placental specimens in cases of congenital infection.[147]

Although the aim of repeated screening was to treat infections detected during pregnancy, this could not be realized in 12 out of 44 cases because infection occurred late in pregnancy. Nine of these 12 cases resulted in congenital infection. Another three congenital infections occurred among children of treated mothers (n=32): two were infected during the second trimester, one during the third trimester. The transmission rate in our series for the treated group was very low (9%), even below that expected on the basis of other studies (19%).[146] There is a considerable difference with the transmission rate for the untreated group, which is very high (75%).
The outcome supports the view that treatment during pregnancy is a positive contribution to prevention of congenital toxoplasma infection. Nevertheless these data are not sufficient to evaluate the effect of treatment since the total number of infections was too small, the treated and untreated groups were not formed by randomization and all untreated infections occurred in the third trimester when a higher transmission rate is to be expected; no cases of infection acquired during the first trimester are represented in the study because women usually did not enter the study before the end of the first trimester.

The TIP study did not include prophylactic treatment of infected children. Treatment would have been advised only for cases of evident acute infection in neonates in whom parasitic activity could be expected. No such cases occurred. There was only one case in which maternal infection occurred shortly before delivery (as proven by the presence of IgM and only a marginal quantity of IgG antibodies in the cord sample (see 4.3 case histories, patient III_1)), that could have benefitted from treatment.
Although the number of congenital infections was low in our series, the fact that no clinical signs were found in the second half year supports our statement that prophylactic treatment might be omitted (see chapter 1).

It was our intention that follow-up care would be provided by the patient's physician and that the study centre would only supply the required information; however, it was noted that the doctor frequently referred the parents

directly to the RIVM or that he did not give the appropriate information. As a result we felt the responsibility, as initiators of the study, for further explanation and information if the parents requested it.
In addition, to relieve practising doctors who seldom encounter congenital toxoplasma infections, we advocate direct accessibility of the study centre for (parents of) the patients. This may be of even greater importance when prolonged follow-up is required to observe the course of subclinical infections. Such a follow-up should include repeated ophthalmological examinations and surveillance of the function of the central nervous system, including intellectual and psychomotoric development. Apart from the very important information that can be obtained from extended follow-up, the procedure offers the best opportunity to support and counsel the parents involved, who envisage the threat of later complications of congenital toxoplasmosis. Annual paediatric and ophthalmological consultations are proposed.

5.7 CONCLUSIONS

Twelve children had a congenital infection due to toxoplasma infection in 43 mothers during their pregnancy; this represents a transmission rate of 28%. The data are not adequate to evaluate the effect of maternal treatment on the risk of infection of the child, but they do suggest a beneficial effect. The mothers of 9 of the 12 infected children had not been treated, 3 had received therapy. In two children a chorioretinitis scar was seen in the left eye within the first year of life, one had intracerebral calcifications. The results do not suggest that it was wrong to omit treatment of children of infected mothers. No serious sequelae became apparent during the second half year of life. In addition a chorioretinitis lesion appeared in one child infection was serologically diagnosed before, during the second year of continued follow-up.
It was very difficult to obtain follow-up data on all children of infected mothers. Motivation of the parents may be enhanced if one counsellor is available to inform and support them during the long-term follow-up study. Frequent comparison of the antibody levels in mother and child and in the course of time in the child itself is the best way to identify congenital toxoplasma infections.

6 SEROPREVALENCE AND FORCE OF INFECTION

6.1 INTRODUCTION

Traditionally the epidemiology of infectious diseases is studied by analysis of incidence figures derived from a notification system. Since the seventies, serological surveillance data have become increasingly important in epidemiological research. It became possible to take into account the susceptibility to disease in different age groups of the population. Today serological data play a major role in the development and evaluation of vaccination strategies for childhood diseases.[69] Even more important, serological models can be applied to diseases that frequently occur subclinically and thus are not found in a notification system.
Few prospective studies to assess the incidence of primary toxoplasma infections and the force of infection are available. In the Netherlands as well as other countries, estimates of the expected number of infections are derived from age-specific seroprevalence data collected in cross-sectional studies.[68,70,194] These data are analysed and interpreted longitudinally as if a group of persons had been followed and repeatedly tested in the course of time. Age-specific seroprevalence, the fraction of a group with specific antibodies as a sign of prior infection, is determined and the rise in seroprevalence with age gives the age-specific risk of acquiring infection.

In order to evaluate the extent of the problem of congenital toxoplasmosis in the Netherlands, the Dutch Health Council relied on such studies, especially that of van der Veen et al.[71] The risk of infection for seronegative women in different age groups and the incidence of primary infection during pregnancy were calculated from seroprevalence data. This resulted in a mean of 12-13 expected infections per one thousand pregnancies.[71,102]
In a prospective study of 28,000 pregnant women, the effects of both primary and secondary preventive measures were investigated; 44 primary infections were traced (see chapter 4), whereas 350 infections (12.5*28) were expected.

The question arose whether the incidence had dropped because of the preventive measures or because of a decreased force of infection during the study period. Since no controls were included in this study, the observed incidence had to be compared with estimates of the incidence used until that time. Another consideration, however, was the accuracy of those estimates. Since they were derived from only one study of 1661 individuals, we applied a model similar to that used by van der Veen to serological profiles obtained in other cross-sectional studies carried out in the Netherlands in order to verify the validity and reproducibility of the estimates. None of the studies involved was designed specifically for this investigation; there are differences in population, time and place.

Our key questions were:
What is the probability of toxoplasma infection during pregnancy for a seronegative woman and what is the resulting annual incidence of primary toxoplasma infection during pregnancy in the Netherlands?

6.2 MATHEMATICAL MODEL

The mathematical model described in this section is based on the following assumptions:
- presence of antibodies indicates that a person has already been infected and that defense mechanisms (cellular and humoral) will prevent re-infection;
- absence of antibodies means that there is no sign of earlier infection, i.e. no sign of immunity; a primary infection might therefore occur;
- increase in seroprevalence in successive age groups is caused by infections that have occurred in the corresponding interval between the age groups.

From the fractions of seropositive women in successive age groups, the force of infection of *Toxoplasma gondii* during the intervals between these age groups can be calculated.[63] The force of infection in the interval between two successive age groups (A_i, A_{i+1}) equals

$$\lambda_{i,i+1}=\frac{\ln(1-f_i)-\ln(1-f_{i+1})}{A_{i+1}-A_i}, \qquad (1)$$

where A_i= middle of age group i (i = 1,2, . . ., n-1) and f_i = fraction of seropositive women at age A_i.[c]

The term force of infection, λ, is synonymous with the intensity of infection; it can be defined as the mean number of contaminations per person per unit of time (year). Here contamination means that enough infectious material is received by a susceptible person to cause infection.

The probability of infection during pregnancy (i.e. per 9 months) of a non-immune woman in age interval (A_i, A_{i+1}) is

$$p_{i,i+1}=1-e^{-9\lambda_{i,i+1}/12}. \qquad (2)$$

Using equation (2) and estimates of the number of non-immune pregnant women in the age intervals (A_i, A_{i+1}) (i = 1,2, . . ., n-1), the number of pregnancies in which a toxoplasma infection occurs can be calculated.
The mean fraction of women in age interval (A_i, A_{i+1}) who have ever developed a toxoplasma infection is

$$\bar{f}_i=(f_i+f_{i+1})/2. \qquad (3)$$

Let N be the total number of pregnant women in a given year and αiN the number of women in age interval (A_i, A_{i+1}), then the number of non-immune pregnant women in the interval (A_i, A_{i+1}) is

$$(1-\bar{f}_i)\,\alpha_i N. \qquad (4)$$

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[c] Equation (1) is in essence the model of van der Veen et al. Equation (2)-(6) are due to J.A.M. van Druten (Department of Medial Statistics, MSA, University of Nijmegen).

From (2) and (4) it follows that in the interval between age group A_i and A_{i+1},

$$\left(1-e^{9\lambda_{i,i+1}/12}\right)\left(1-\overline{f}_i\right)\alpha_i N \tag{5}$$

pregnant primarily infected women can be expected. It immediately follows that the total number of primary infections in the whole age range is

$$\sum_{i=1}^{n-1}\left(1-e^{9\lambda_{i,i+1}/12}\right)\left(1-\overline{f}_i\right)\alpha_i N. \tag{6}$$

The calculations in this study are standardized to 170,000 births per year and adjusted to the age distribution of pregnant women in the Netherlands in 1975, according to the Health Council report (source:CBS). This standardization makes it possible to compare the estimates in this study with the estimates presented in the report of the Health Council.[102]

6.2.1 SEROPREVALENCE 1972-1976, TILBURG

During the period 1972-1976 1661 sera, representing 12 age groups (0-79 year), were collected at the St.Elisabeth Hospital in Tilburg (province North Brabant) for diagnosis of viral respiratory disease; they were also tested for specific antibodies against *Toxoplasma gondii*. With an indirect immunofluorescent assay (IIF) IgG antibodies were assessed. The age-specific seroprevalence data from the Tilburg study, as presented in the Health Council report, are given in table 6.1 and figure 6.1. In the same table and in figure 6.2 the probability of a toxoplasma infection during pregnancy for a seronegative woman, derived from these seroprevalence data and equation (2), is given. For women aged 17.5 to 35 years, this probability is independent of age (2.2%); in the older age groups the probability decreases. Estimates of the number of primarily infected pregnant women per age interval are given in table 6.1 as well.

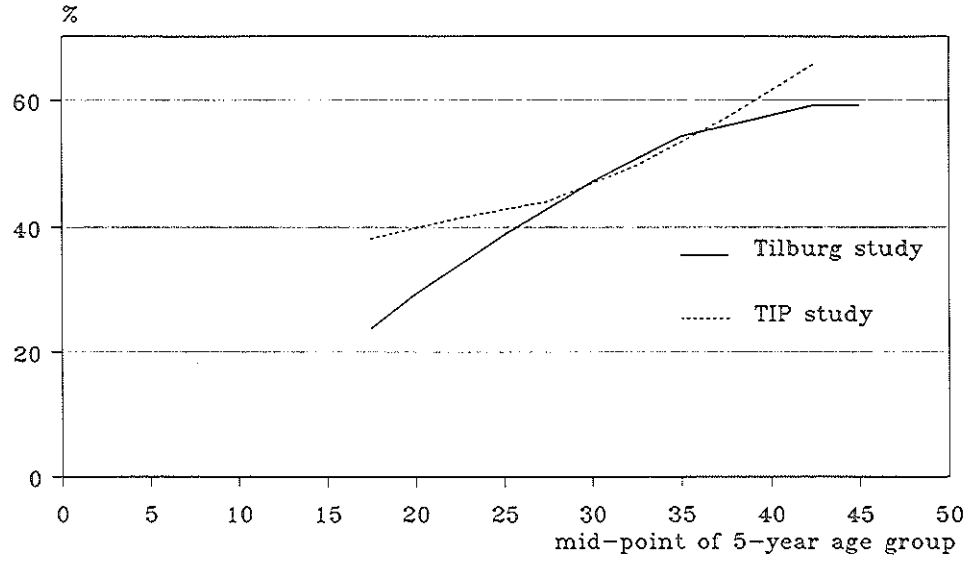


Figure 6.1 Age-specific seroprevalence (%) in the TIP and the Tilburg study

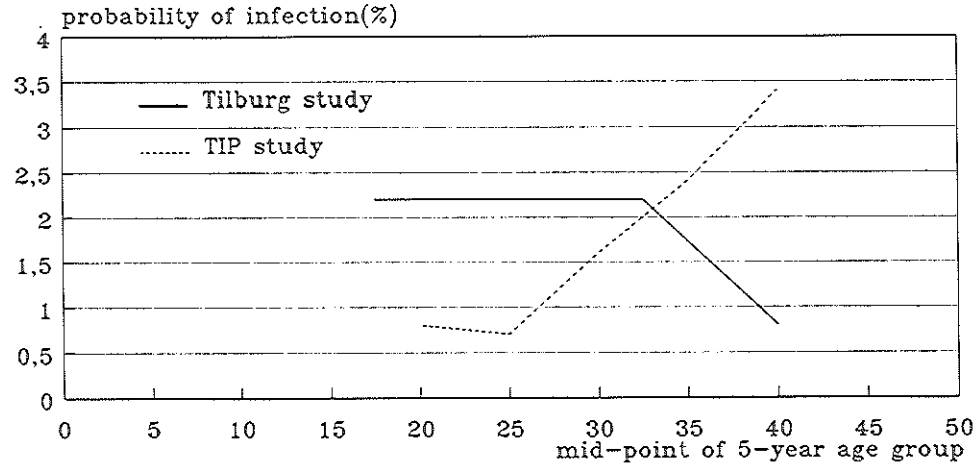


Figure 6.2 Probability (%) of a toxoplasma infection during pregnancy for a seronegative woman in the time interval between two successive age groups; derived from data of the TIP study and the Tilburg study

Theoretically (equation (6)) 2,100 infections can be expected per 170,000 pregnancies, i.e 12.4 per 1000 pregnancies. The estimates in table 6.1 differ slightly from the estimates listed in the Health Council report because the latter are based on approximate calculations.

Table 6.1 Seroprevalence (%) at age A_i (middle of the age group), the probability of a toxoplasma infection during pregnancy for a seronegative woman in age interval (A_i, A_{i+1}) and estimates of the annual number of pregnant women with a primary toxoplasma infection per age interval; based on data from the Tilburg study.*

age A_i (year)*	seroprevalence (%)*	probability of infection (%)**	number of primary infections***
17.5	23.8		
20.0	29.2	2.2	112
25.0	38.8	2.2	737
30.0	47.1	2.2	942
35.0	54.3	2.2	275
45.0	59.2	0.8	34
total			2,100

* presented in the Health Council report[102]

** equation (2); the probability is calculated for the interval between the midpoints of two successive age groups (A_i and A_{i+1})

*** equation (5); the probability is calculated for the interval between the midpoints of two successive age groups (A_i and A_{i+1})

6.2.2 SEROPREVALENCE 1987/1988, SOUTH HOLLAND

During the TIP study, from March 1987 to March 1988, 27,967 pregnant women in South Holland were tested for specific anti-Toxoplasma antibodies (IgG) with an enzyme-linked immunosorbent assay (ELISA). The seroprevalence data of this study were described in chapter 4 (see table 4.1); the data on women in the age groups 15-19, 20-24, . . ., 40-44 years are presented in table 6.2 and figure 6.1; 27,949 women fell within this age range.

The probability of a toxoplasma infection during pregnancy for a seronegative woman, as calculated from equation (2), is given in the same table and figure 6.2. The mean probability for the age groups is about 2%, which compares with the probability for the Tilburg population. It is striking that the probability of infection for women up to the age of 30 years is lower than that derived from the Tilburg data; however, above the age of 30 years, it tends to increase while it decreased according to the Tilburg data. Since most of the seronegative women are in the younger groups the effect is that the annual number of infections during pregnancy decreases to 1,095 per 170,000 pregnancies, i.e. 6.4 per 1000 women (table 6.2). This estimate is about 50% lower than the estimate derived from the Tilburg data.

Table 6.2 Seroprevalence (%) at age A_i (middle of the age group), the probability of a toxoplasma infection during pregnancy for a seronegative woman in age interval (A_i, A_{i+1}) and estimates of the annual number of pregnant women with a primary toxoplasma infection per age interval; based on data from the TIP study.

age A_i (year)	seroprevalence (%)	probability of infection (%)*	number of primary infections**
17.5	38.2		
22.5	41.5	0.8	162
27.5	44.1	0.7	251
32.5	49.7	1.6	426
37.5	57.1	2.4	166
42.5	65.8	3.4	90
total			1,095

* equation (2); the probability is calculated for the interval between the midpoints of two successive age groups (A_i and A_{i+1})

** equation (5); the probability is calculated for the interval between the midpoints of two successive age groups (A_i and A_{i+1})

6.2.3 SEROPREVALENCE 1980 AND 1985, SENTINEL STUDY

A network of general practitioners (GP) throughout the country, whose

patients represent 1% of the Dutch population, participates in a continuous Sentinel Survey. To obtain samples from a representative part of the Dutch population, the 45 physicians of these sentinel practices were asked to take blood from 24 healthy persons in their practice, one man and one woman from each of the following age groups: 10-14, 15-19, . . .,60-64, ≥65 years. The RIVM was asked to organize the study, that is to be repeated every 5 years. The samples were tested serologically for antibodies against measles, rubella, mumps, polio type 1, 2 and 3, diphtheria and tetanus. The Laboratory for Parasitology and Mycology was allowed to use this serum bank; unfortunately the amount of available serum was often insufficient for testing.

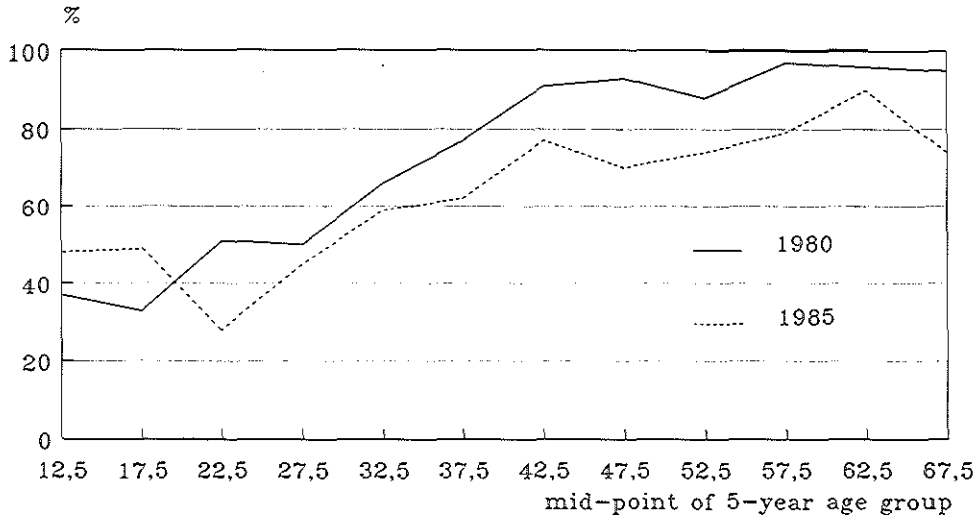


Figure 6.3 Age-specific seroprevalence (%) in the sentinel studies (1980 and 1985 respectively)

In 1980 464 samples and in 1985 693 samples were assessed with an IgG ELISA for *Toxoplasma gondii*. The results are given in table 6.3 and figure 6.3. The seroprevalence level found for the older age groups is clearly higher in the 1985 study. Because of the small number of persons examined in the different age groups (interval: 5 years), the age-specific seroprevalence curve has an irregular shape. Application of the mathematical model requires that seroprevalence be a non-decreasing function of age (otherwise a 'negative' probability of a toxoplasma infection would be represented). In order to overcome this difficulty, the successive age groups were combined. The

seroprevalence for the combined age groups 15-24, 25-34, . . ., 45-54 years is given in table 6.4. However, the problem was not solved for the 17.5-20 year interval for the 1980 data. The prevalence was 49% at 17.5 years (mid-point of age group 15-19 year) and 37% at 20 years. To avoid a 'negative' infection risk in the interval 17.5-20 years, a seroprevalence of 37% was assigned to the age of 17.5 years.
Consequently the probability of a primary infection in the interval between the ages of 17.5 and 20 years is zero, as is the number of primary infections during pregnancy. For the 1985 study the successive age groups were combined in a similar way; a further adaptation as described above was not necessary. Data on individuals under 15 years and equal to or above 55 years were excluded. The intervals between the age groups for which the probability of infection was calculated are 17.5-20, 20-30, 30-40, 40-50 years (see table 6.4). For those aged 20-30 years the probability is 2.0% and 2.3% for the 1980 and 1985 groups, respectively. For 30-40 year-old women the 1985 data give a substantially higher probability than the 1980 data. This is in line with the significantly higher seroprevalence found for 35-44 year-old individuals in 1985 versus 1980 (chi-square test for comparison of proportions; p-value <0.05).

Table 6.3 Age-specific seroprevalence (IgG/ELISA); sentinel study 1980 and 1985.

	1980		1985	
age groups (years)	number examined	seropositive (%)	number examined	seropositive (%)
10-14	29	48	43	37
15-19	37	49	60	33
20-24	47	28	59	51
25-29	44	45	62	50
30-34	39	59	58	66
35-39	39	62	62	77
40-44	39	77	57	91
45-49	37	70	55	93
50-54	39	74	60	88
55-59	42	79	58	97
60-64	30	90	54	96
≥ 65	42	74	65	95
total	464	62	693	73

Table 6.4 Age-specific seroprevalence calculated from the data of the sentinel study for combined age groups, the probability of a toxoplasma infection during pregnancy for a seronegative woman in age interval (A_i, A_{i+1}) and estimates of the annual number of pregnant women with a primary toxoplasma infection per age interval.

	1980			1985		
age A_i (year)	seropreval. (%)	probability of infection**	number of primary infections***	seropreval. (%)	probability of infection**	number of primary infections***
7.5	37*	0.0*	0*	33	4.1	178
20	37	2.0	1,425	42	2.3	1,481
30	52	3.3	398	58	7.1	630
40	69	0.8	11	84	3.7	23
50	72			90		
total			1,834			2,312

* assigned value, see text

** equation (2); the probability is calculated for the interval between the midpoints of two successive age groups (A_i and A_{i+1})

*** equation (5); the probability is calculated for the interval between the midpoints of two successive age groups (A_i and A_{i+1})

Estimates of the annual number of primarily infected pregnant women per age group, derived from the sentinel studies, are also given in table 6.4. The number of women at risk was smaller in 1985, the probability of a primary infection during pregnancy for a seronegative woman was, however, higher (table 6.4). The estimate of the number of primary infections, which is influenced by both of these factors, turns out to be higher for the 1985 data (2,312 versus 1,834 for 1980).

In addition to longitudinal interpretation and analysis of each cross-sectional study, a cross-over analysis can be performed. In a cross-over analysis the seroprevalence for age group A_i in 1980 is compared with the seroprevalence for the age group five years older in 1985, A_{i+1}. To estimate the force of infection and the probability of infection for a seronegative woman during

pregnancy in the years 1980-1985 we can apply the mathematical model described in section 6.2.
Cross-over analysis compares data on the same birth cohort, while a longitudinal analysis of a cross-sectional study compares data on different birth cohorts. Given representative serum samples tested with the same serological assay, cross-over analysis of the data from repeated cross-sectional studies approaches the ideal of a prospective study.
Table 6.5 presents the cross-over seroprevalence data from the sentinel studies in 1980 and 1985. The seroprevalence was 49% for 15-19 year-olds (mid-point 17.5 years) in 1980; five years later, in 1985, the seroprevalence was 51% for the 20-24 year-old individuals (mid-point 22.5 years). These data do not fit the serological profile for the other age groups.

Table 6.5 Seroprevalence (%) at age A_i in 1980 and at age A_{i+1} in 1985, the probability of a toxoplasma infection during pregnancy for a seronegative women in the age interval (A_i, A_{i+1}) during the period 1980-1985 as derived from cross-over analysis and estimates of the annual number of pregnant women with a primary toxoplasma infection per age interval; based on the data from the sentinel studies (table 6.3).

age interval (A_i, A_{i+1})	seroprevalence (%) at A_i 1980	seroprevalence (%) at A_{i+1} 1985	probability of infection*	number of primary infections**
17.5-22.5	49	51	0.7	108
22.5-27.5	28	50	5.4	2,113
27.5-32.5	45	66	6.6	1,509
32.5-37.5	59	77	8.6	412
37.5-42.5	62	91	19.9	327
total				4,469

* equation (2)
** equation (5)

The seroprevalence figures 49% (1980) and 51% (1985) found for the first cohort are quite different from those calculated for the other birth cohorts and are to be interpreted with caution. Table 6.5 also gives the probability

of infection during pregnancy for a seronegative woman and estimates of the annual number of primary infections during pregnancy.
Cross-over analysis apparently results in a very high probability of infection for individuals 20 years or older in 1980.
The incidence of primary infections clearly exceeds the incidence derived from cross-sectional analysis: 4469 or 26 per 1000. If we take into account the fact that the incidence in the age interval 17.5-22.5 is probably underestimated, the incidence of primary infections might have been 30 per 1000 pregnancies in the period 1980-1985.

6.2.4 SEROPREVALENCE 1982-1987, ENSCHEDE

A repeated cross-sectional serological survey was carried out using blood samples from pregnant women from Enschede and surroundings.[d] The blood samples were collected to screen for hepatitis B during the period 1982-1987. We were able to determine the toxoplasma seroprevalence rate for samples randomly taken from that serum bank (stratified for the years 1982 to 1987). The sample is slightly biased since the blood samples of hepatitis B positive women were no longer available. In total 2570 serum samples were tested with an ELISA for specific antibodies (IgG) against *Toxoplasma gondii*. Table 6.6 shows the age-specific seroprevalence in 1982-1987.

The age-specific seroprevalence rates are considerably lower than those found in the TIP study (Table 6.2). The expected gradual rise in seroprevalence with age is not demonstrated. Women between 15 and 25 years of age exhibit hardly any increase whereas the seroprevalence for those over 30 years of age rises quickly to a high level. To examine the relationship between time and age-specific seroprevalence rates a chi-square test (for a 2x6 table) and the trend test of van Eeden were applied.[195] In two age groups, 20-24 and 30-34 years, there is a declining trend in seroprevalence over the years 1982 to 1987. Although the samples were taken at random, the serological patterns are quite unstable and the results difficult to interpret. This can partly be ascribed to the relatively small number of samples per age/year group. One should hesitate to draw conclusions from these data. An uneven increase

[d] Enschede is a medium-sized town of around 250,000 inhabitants in the province Overijssel.

in prevalence over time indicates a possible cohort effect instead of merely the effect of ageing.

Table 6.6 Seroprevalence (IgG/ELISA) in the Enschede region (1982-1987); percentage IgG-positive women according to age and year of sampling.

year of sampling	seroprevalence by age group (year)				
	15-19	20-24	25-29	30-34	35-39
1982	35.5	45.3	33.8	50.8	50.0
1983	25.3	38.1	37.6	55.0	50.4
1984	34.5	29.9	29.7	39.3	53.6
1985	27.3	27.1	23.6	33.3	53.5
1986	31.0	26.0	30.0	37.0	44.0
1987	32.3	30.3	38.4	32.8	44.4
total	31.0	32.8	32.2	41.4	49.3
	p-values				
χ^2-test	n.s.	(*)	n.s.	**	n.s.
trendtest	n.s.	**	n.s.	***	n.s.

n.s. $p > 0.10$; not significant
(*) $0.05 < p \leq 0.10$
* $0.01 < p \leq 0.05$
** $0.001 < p \leq 0.01$
*** $p \leq 0.001$

To evaluate eventual cohort effects the seroprevalence data are grouped according to birth cohort instead of age in table 6.7. It is remarkable that the seroprevalence level for the 1948-1952 cohort is much higher than those for the other cohorts. The difference in seroprevalence between 1982 and 1987 for each cohort reveals no effect of five years of ageing.
Actually the prevalence in 1982 was always higher than in 1987.
This suggestion of cohort effects rather than ageing effects prevents us from

using the mathematical model. As described in section 6.2 the model is based on the assumption that an increase in seroprevalence as a function of age can be attributed to age effects and not cohort effects.

Table 6.7 Seroprevalence (IgG/ELISA) in the Enschede region (1982-1987); percentage IgG-positive women per birth cohort and year of sampling.

year of sampling	prevalence per birth cohort			
	'48-'52	'53-'57	'58-'62	'63-'67
1982	50.8	33.8	45.3	35.6
1983	51.4	43.1	32.7	34.0
1984	42.7	34.3	28.8	33.3
1985	45.9	25.4	24.2	25.4
1986	47.0	32.0	29.0	30.0
1987	44.4	32.7	38.4	30.3
total	47.0	33.6	33.1	31.4
	p-values			
χ^2 test	n.s.	n.s.	(*)	n.s.
trend test	n.s.	n.s.	n.s.	n.s.

n.s. $p > 0.10$; not significant
(*) $0.05 < p \leq 0.10$

6.3 ESTIMATION OF THE FORCE OF INFECTION FROM THE TIP STUDY

The risk of acquiring a toxoplasma infection can also be derived from prospective studies. In such a study of 15,170 seronegative women, 15,166 of whom were between 15 and 45 years old, 44 primary toxoplasma infections were recognized by the demonstration of seroconversion (chapter 4). Table 6.8 presents the age distribution of the 44 cases and the percentage of primary infections among seronegative pregnant women in these age groups.

Table 6.8 Age distribution of seronegative women and the number and percentage of primary infections during pregnancy (prospective study).

age interval A_i, A_{i+1} (years)	number of seronegative women	primary infections during pregnancy*	
		number	percentage
15.0-22.5	2,176	5	0.23
22.5-27.5	5,154	12	0.23
27.5-32.5	5,260	18	0.34
32.5-37.5	2,168	8	0.37
37.5-45.0	408	1	0.25
total	15,166	44	0.29

* This number probably differs from the true number of infections. See text.

For the purpose of comparison with the estimates presented in table 6.2, the age distribution used here differs from that presented in chapter 4 (table 4.2). The percentage of primary infections ranges between 0.23 and 0.37%. This is clearly much lower than the model-based estimates of the probability of a primary toxoplasma infection derived from cross-sectional data (table 6.2). The suggested increase in the chance of infection with age, previously mentioned in section 4.4 (table 4.2), is not statistically significant according to the trend test of van Eeden (one-sided p-value 0.17)[c] using the age classification of table 6.8. It is realistic to state that the number of infections traced during the TIP study does not represent all infections that occurred in the pregnant women.
In prospective studies it is unfortunately inevitable that subjects are lost during follow-up. This study too encountered missing values and missing subjects. In order to estimate the probability of a primary infection during pregnancy the percentage of infections must be adjusted.
It is assumed that there is no relationship between missing values and the occurrence of a toxoplasma infection. We may therefore correct for the

[c] In the trend test the age groups 32.5-37.5 and 37.5-45.0 were combined to one age group.

number of missed infections in the following way. The mean duration of observation was approximately 6 months per women. Correcting for limited observation (6 months instead of 9 months) increases the probability of infection during pregnancy (0.29%, table 6.8) to 0.44%. Since there is a time lag after the moment of infection before antibodies reach a detectable level, some late infections may have been missed at the last blood sampling, namely at birth of the child. Therefore considering all factors involved we assume that the probability of contracting a primary infection during pregnancy in the TIP study was about 0.5%. The annual force of infection then is approximately $12(0.005/9) = 0.007$.

6.4 DISCUSSION

The results obtained from the various studies are compared below. Table 6.9 provides a summary of the model-based estimates of the number of seronegative pregnant women and the number of pregnant women with a primary toxoplasma infection (based on the seroprevalence data from the Tilburg study, the TIP study and the two sentinel studies).

Table 6.9 Model-based estimates of the number of seronegative pregnant women and the number of pregnant women with a primary toxoplasma infection, derived from seroprevalence data of the Tilburg study, the TIP study and the sentinel studies 1980 and 1985, respectively (per 170,000 pregnancies per year).

study	seronegative women		primary infections	
	number	%	number	per 1000
Tilburg	99,477	59	2,100	12
TIP	93,215	55	1,095	6
sentinel '80	89,032	52	1,834	11
sentinel '85	78,162	46	2,312	14

The model-based estimates of the probability of toxoplasma infection during pregnancy of a seronegative woman of the TIP study is quite different from the estimates based on the Tilburg study or the sentinel studies. This difference

cannot be attributed to a different method of analysis but to differences in study design. The TIP study, the Tilburg study and the sentinel studies differ in four aspects:

1. the period of data collection: TIP: March 1987-March 1988; Tilburg: 1972-1976; sentinel studies: 1980 and 1985.
2. the study population: TIP: pregnant women; Tilburg: men and women suspected of viral respiratory disease; sentinel studies: healthy men and women.
3. the serological technique: TIP: IgG/ELISA; Tilburg: IgG/IIF; sentinel studies: IgG/ELISA.
4. the place: TIP: province South Holland; Tilburg: a region in the middle of North Brabant; sentinel studies: all over the country.

Each of these aspects may have influenced the serological profiles and thus may have led to different estimates. With respect to the different serological profiles (figures 6.1 and 6.3), the following comments can be made:

1. The seroprevalence in the TIP study increased sharply in the interval between 32.5 and 42.5 years. These individuals were born between 1945 and 1955. A relatively high force of infection in that period may have been responsible for the high seroprevalence found for the age group 32.5-42.5 years. The Enschede study also suggests possible cohort effects (birth cohort 1948-1952). This leads to the conclusion that the factor time, i.e. cohort effects in addition to or instead of effects of ageing, should be considered in future research.
2. It is assumed that serological profiles for men and women are very similar and that differences between pregnant and non-pregnant women may be neglected. The sentinel studies, however, indicated some differences between men and women. Further research is needed to investigate the role of gender and/or parity.
3. The techniques used in serology may be responsible for some of the differences. Van der Veen et al. hypothesized and van Druten et al. theorized that infected individuals remain seropositive, when tested with the IIF, for a long-lasting but not ever-lasting period.[63,71] The flattening of the Tilburg seroprevalence curve is probably a consequence of a balance between seroconversion and seroreversion in the older age groups. Therefore the true number of individuals ever infected may be substantially higher than that derived from the IIF curve. This is in line

with the observation that the ELISA is more sensitive than the IIF. The TIP study only covers the age range 17.5-45 years. The sentinel studies included older people and also used ELISA tests; these studies showed a substantially higher level of seroprevalence for the older age groups.

4. Regional differences are considered to be of lesser importance in the Netherlands but this cannot be properly assessed from the data presented here. In the past we carried out a study using the IIF to test blood samples collected on filtre paper to compare the results obtained for South Holland and North Brabant. The results showed that spatial differences do exist.[196]

It is interesting to compare the figures of the prospective study with the model-based estimates derived from the cross-sectional studies. As indicated by the variations in the estimates, none of them may be accepted as a definitive value that characterizes the epidemiological situation in the Netherlands. All cross-sectional studies are actually retrospective studies: the fraction of individuals who have experienced a toxoplasma infection once before in their lives is assessed. This also applies for the seroprevalence data of the TIP study. However, since the TIP study is a direct investigation of the target population to determine the incidence of congenital toxoplasmosis, the number of participating women was very high (75% of all pregnancies) and the study region represents more than 15% of the total Dutch population of pregnant women, the estimates obtained from analysis of the TIP seroprevalence data are probably the most reliable.
However, the percentage of primary infections traced during the prospective study differs considerably from these estimates (cf. table 6.2 and 6.8).
Even after correction for the limited period of observation during pregnancy, the probability of acquiring a primary toxoplasma infection during pregnancy estimated from the prospective study (0.5%) differs markedly from the probability estimated from cross-sectional studies (2%).
Apart from the design of the studies and the method of analysis, there is one other essential difference between the studies: during the prospective study preventive measures were applied. If these preventive measures had been applied throughout the country, the number of primary infections established in the TIP study provisionally could be used to estimate the "minimum" annual number of infections in the Netherlands. Firstly the 44 primary infections should be multiplied by a factor 1.5 for limited observation, resulting in 66 infections among 27,949 women (2.4/1000). An

approximate 95% confidence interval can be obtained if we use a Poisson approximation of the probability distribution of the observed 44 primary infections ($44 \pm 2\sqrt{44}$). For the country as a whole this would amount to 400 ± 120 primary infections per 170,000 pregnancies (assuming that South Holland is representative for the Netherlands).
Obviously prospective investigations yield the most reliable results, and the TIP study gives a good impression of the extent of the problem of primary infections during pregnancy when prevention is applied. It is difficult, however, to quantify the effect of intervention. Since no prospective studies without intervention have been performed in our country, the number of infections encountered during the prospective intervention study can only be compared with model-based estimates derived from seroprevalence data, using utmost caution. The pitfalls in estimating the risk of infection from seroprevalence data and the incidence of primary infections during pregnancy have been clearly underlined in this chapter.

6.5 SUMMARY

A prospective preventive study was set up because previous estimates of the extent of the problem of congenital toxoplasmosis in the Netherlands were alarming, the reliability of these estimates now has to be questioned. Longitudinal interpretation of age-specific seroprevalence data from different studies does not lead to either unequivocal estimates of the probability of primary toxoplasma infections or unbiased estimates of the incidence of primary infections. None of the seroprevalence studies presented here was designed for this particular purpose; different study populations from different places in different periods and sometimes different tests, are involved. This hampers interpretation of the results.
The discrepancy between the serological profiles could be attributable to these different factors. At present it is not clear which profile represents the Dutch population as a whole: the actual number of primary infections remains uncertain. Moreover, one may ask whether the conditions for longitudinal interpretation of these cross-sectional data were met.
Bearing in mind the limitations of cross-sectional study designs, we may provisionally use the following values to describe the incidence of primary toxoplasma infections during pregnancy in the Netherlands. The probability of a toxoplasma infection for a seronegative woman – as derived from the seroprevalence data of the Tilburg study and the TIP study – is approximately

2%. The incidence of primary infections per 1000 pregnant women, estimated from these two studies, is 12 and 6 per 1000, respectively. We should consider these estimates with reserve. It may be that the real incidence is substantially higher. Cross-over analysis of the data of the sentinel study suggests that the incidence could amount to 30 primary infections per 1000 pregnancies. The prospective TIP study on the other hand shows that – if prevention is applied – the incidence may be as low as 2 per 1000. From the above one may deduce that the incidence of primary toxoplasma infections is somewhere between 2 and 30 per 1000 pregnancies. The lower value may be obtained if primary prevention is applied. The higher value needs to be confirmed by means of further epidemiological research.
With regard to future research involving seroprevalence studies to monitor epidemiological trends, regular surveillance of the same population using the same method of sampling and the same serological test is a prerequisite.

Prospective assessment of the number of primary toxoplasma infections in pregnant women who were involved in a preventive study yields far fewer infections than any estimate from cross-sectional studies. This may reflect the effect of primary prevention – possibly reinforced by concomitant secondary prevention. No control data are, however, available to prove such an effect. Future prospective studies have to assess whether the incidence stays at this level when merely primary prevention is applied.

7 IGM AS A SCREENING PARAMETER FOR TOXOPLASMA INFECTIONS DURING PREGNANCY?

7.1 INTRODUCTION

Infections with *Toxoplasma gondii* are predominantly asymptomatic. Yet toxoplasma infections represent an important health problem: if they occur during pregnancy parasites may be transmitted from mother to foetus. This may cause stillbirth or the (premature) birth of a congenitally infected child: only a minority is born with manifest defects, but probably all of the seemingly unaffected children will develop defects in later life, especially visual impairment due to chorioretinitis.[80,86,87]
After infection active parasites, tachyzoites, penetrate cells where they multiply and are dispersed via the bloodstream and lymphatic circulation throughout the body. During this phase of acute infection the defense mechanisms of the host (both cellular and humoral) come into action; offensive and defensive forces gradually become balanced, and the infection becomes latent, i.e. cysts containing many inactive parasites, bradyzoites, lie within tissues of the host. Antiparasitic drugs are only effective against replicating stages, tachyzoites, but do not affect tissue cysts and the numerous slumbering bradyzoites. The term "recent infection" is used to indicate an infection that began only some months ago, thus suggesting that there might still be active replicating tachyzoites that are susceptible to antimicrobial agents.
The need to detect asymptomatic primary infections during pregnancy in time, in order to be able to reduce the risk of infection of the child by treatment of the mother, has led to the increasing use of serodiagnostic techniques for screening purposes.

Primary infections are proven by seroconversion: evidence of production of specific antibodies in a person who recently, at a previous examination, was seronegative. If antibodies are already present in the first blood sample

taken, it is evident that infection with the microbiological organism has already occurred, but it is not known when it occurred. For a pregnant woman, who is not tested for the first time until she is about three months pregnant, this means that it can be neither excluded nor concluded that she acquired the infection around the time of conception or in early pregnancy. In order to get more information a second test can be carried out: a marked rise in antibody titre is suggestive of recent infection. Another possibility that saves time is to assess class-specific antibodies. Generally, specific antibodies of the IgM class are formed at first, followed some time later by antibodies of the IgG class.[197] The classical concept is that IgM antibodies are only produced after a primary infection and may then be found in the blood for some time, while IgG antibodies persist (life)long. As a consequence IgM assessment might be of additional value in prospective investigations since it is the earliest sign of infection; moreover in retrospective investigations it suggests that infection took place recently. Unfortunately, with our very sensitive modern methods, positive IgM results are frequently obtained even when infection actually did not occur recently.[117,198,199] In a group of patients with lymphadenopathy due to toxoplasmosis 20% were still IgM-positive according to an ELISA 12 months later.[118] Nevertheless it has been advocated that IgM tests should be included in a screening programme in order to recognize early infections and allow the women the option of terminating pregnancy.[200]
It is debatable whether the efficacy of serological screening in preventing congenital toxoplasmosis is enhanced by IgM detection (in addition to IgG detection), whether IgM detection identifies recent infections unequivocally and whether it is ethically acceptable to bother healthy pregnant women, i.e. those without any sign of infection, with it.
In order to evaluate the usefulness of IgM as a screening parameter within the scope of prevention, a study of the prevalence of IgM antibodies in pregnant women was performed.

7.2 MATERIALS AND METHODS

For this investigation of the prevalence of specific IgM antibodies a large store of sera obtained during a prospective study of pregnant women could be used. This study, the so-called TIP (Toxoplasma Infection Prevention) study, was carried out in the Netherlands during 1987 and 1988. Primary and secondary preventive measures were applied during pregnancy in order

to reduce the frequency of congenital toxoplasma infections. The participants were advised not to consume raw or undercooked meat, to wash vegetables thoroughly, to clean the cat's box daily and, if possible, to ask someone else to do it, and to avoid contact with soil by wearing gloves when gardening. They were tested for the presence of specific antibodies and – if absent – retested every 8 weeks. If a primary infection was traced by seroconversion, i.e. the appearance of antibodies in an initially seronegative woman, therapy was administered. In view of the above-mentioned considerations, only the presence of IgG was used as a parameter in this screening study. Nearly 30,000 women in the province of South Holland participated; in total over 70,000 blood samples were obtained.

For the assessment of the prevalence of IgM antibodies in healthy pregnant women we took a random sample of the sera collected during the TIP study as follows. The TIP samples were stored in boxes of 80 samples each, in the sequence in which they were sent in. Therefore one box might contain first samples as well as repeat samples from women together with samples from neonates. Every 10^{th} box was taken for additional IgM assessment. Sera of neonates were omitted because they were irrelevant as far as this study was concerned. Thus 5169 sera were available. It is possible that several sera from one woman were included in this series. The numbers of samples and pregnant women in this investigation are presented in the table 7.1. The distribution of first and consecutive sera in the series (37.5% and 62.5%) does not differ from that in the TIP study (38.9% and 61.1%), which indicates randomness of selection.

The IgG status of all sera selected for IgM detection was known. If IgM screening yielded a positive result, the complete series of available samples from that particular woman was tested.

In addition to IgM prevalence for the samples taken from the TIP store of sera, the results of other diagnostic tests of the TIP study are analysed in this chapter. At first examination 45% of pregnant women in the TIP study exhibited IgG antibodies; thus 55%, the seronegative women, were repeatedly screened. The mean duration of observation was 6 months. In 44 cases a primary toxoplasma infection was recognized by seroconversion. In order to confirm seroconversion, two consecutive samples were tested simultaneously; at that moment IgG as well as IgM antibodies were assessed. Subsequently a sample was taken monthly up to the end of the pregnancy; a sample was taken from her child at birth, six months later, one year later and then every year.

An enzyme-linked immunosorbent assay (ELISA) was used to detect specific IgG and IgM antibodies to *Toxoplasma gondii*.[183,184]

Table 7.1 Number of sera and women in the random series

samples*	number of sera	number of women
first samples	1936	1933
repeat samples	3233	2939
total	5169	4649**

* From three women two first samples were available; after an abortion the first sample taken during the new pregnancy was again categorized as a first sample.

** This number is less than the sum of the column since, in some cases, more than one sample came from an individual woman.

7.3 RESULTS

Monthly examination of infected women showed IgM persistence in 16 of 44 women. The duration of persistence of IgM antibodies varied from 2 to 83 weeks, with a mean of 28.06 weeks. Based on these findings an exponential model for persistence was fitted:

$$p(t) = \lambda e^{-\lambda t}$$

where p(t) is the probability that IgM persists at time t, λ describes the decrease in the function with time, and t is time in weeks. A mean persistence of 28 weeks was estimated from the data ($=1/\lambda$). From this model the distribution of the number of women (%) with a persistence beyond t weeks can be derived; see figure 7.1. It can be seen that in 35% of the study population IgM persists for less than 12 weeks, in 50% for less than 19.45 weeks, in 85% for less than 53.24 weeks. This means that in more than 15% of cases IgM persists for more than one year.

When assessment of IgG in the TIP study revealed a possible primary infection, this suspicion had to be confirmed in a simultaneous diagnostic test of two consecutive samples. The number of women with IgM in the IgG-negative sample preceding seroconversion was counted: 13 of the 44 infected women. If IgM detection had been included in the screening protocol,

these infections would have been recognized earlier, therapeutic intervention could have been started sooner and therapy consequently might have been more effective.

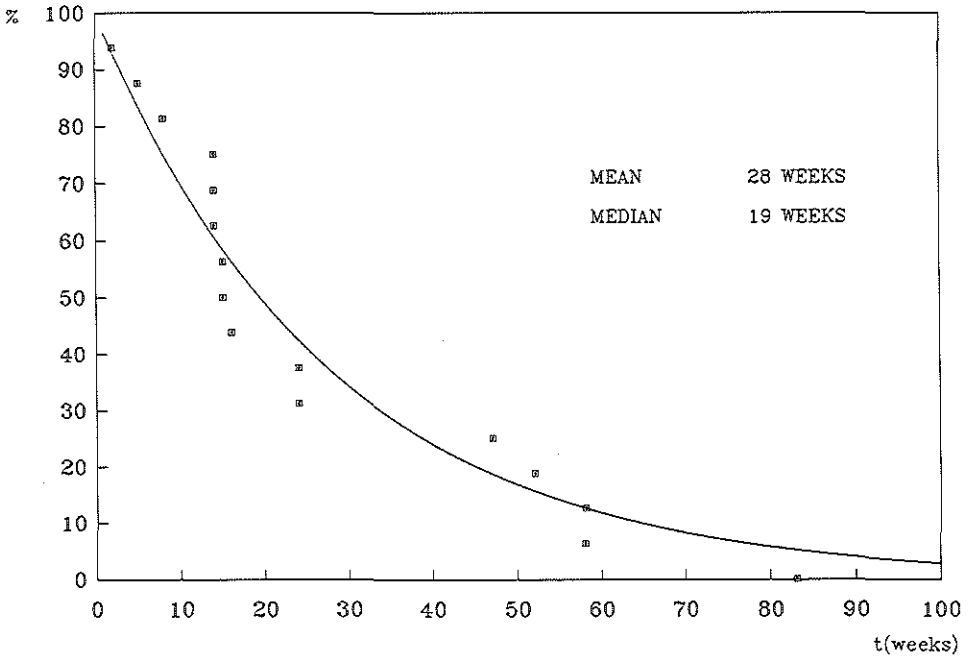


Figure 7.1 Percentage of persons with specific IgM antibodies persisting beyond t weeks after infection

Table 7.2 presents the results of the IgM investigation of randomly selected samples; the IgG results of TIP screening are also given.
In total 129 of the 1936 first samples were IgM-positive, i.e. a prevalence of 6.7%; the prevalence of IgM in repeat samples (2.3%) is strongly biased since all samples came from IgG-negative women.
IgG was present in 849 of the 1936 first samples (44%; 95% confidence interval 42-46%). From this seroprevalence the prevalence of prior toxoplasma infections in the population of pregnant women in the Netherlands can be estimated.

Table 7.2 Number of samples (first samples and repeat samples) with specific IgG and IgM antibodies.

		IgG^+	IgG^-	total
first samples	IgM^+	109	20	129
	IgM^-	740	1067	1807
	subtotal	849	1087	1936
repeat	IgM^+	4	70	74
samples	IgM^-	37	3122	3159
	subtotal	41	3192	3233
	total	890	4279	5169

The 90 IgM-positive/IgG-negative samples in the IgM study were taken from 85 women. The result of the simultaneous examination of all samples from each woman was as follows:

- no more serum available for re-examination — 2 women
- presence of IgM antibodies in the index serum not confirmed; other sera negative as well — 14 women
- presence of IgM antibodies in the index serum confirmed at re-examination; no additional sera available — 2 women
- persistence of IgM in all sera, the number of samples per women being
  - 7 times 2 samples
  - 12 times 3 samples
  - 33 times 4 samples
  - 11 times 5 samples
  - 1 time 6 samples

  — 64 women
- incidental occurrence of IgM — 3 women

None of the 85 women seroconverted; all samples remained seronegative according to the IgG test.
Fourteen of the 90 samples were not IgM-positive at re-examination, i.e. 15.5%; 64 out of 85 women showed persistence of antibodies. Using the median values for the duration of the intervals between consecutive samples in the TIP study and the number of IgM-positive samples for each of the

64 women, a median value for the persistence of 19 weeks was estimated. This estimate of the persistence is limited by the period of observation.

7.4 FURTHER MATHEMATICAL ANALYSIS

The results will be analysed and discussed separately from the standpoint of the retrospective recognition of recent infection and that of the prospective recognition of acute infection.

7.4.1 SPECIFIC IGM ANTIBODIES: INDICATOR OF RECENT INFECTION

From prospective examinations of the 44 patients who became infected with *Toxoplasma gondii*, it is already evident that IgM antibodies persist too long to play a decisive role in the discrimination between recent and latent infections. If the first examination of pregnant women is done in the 12th week of gestation (median value for the first sample in the TIP study) and they are screened for IgM antibodies as well, then the previous result (IgM persistence for less than 12 weeks in 35% of cases) leads to the conclusion that only 35% of the IgM-positive women will have been infected around the time of conception or in early pregnancy; 65% of these women will have acquired the infection earlier, probably without risk for the current pregnancy.

The IgM prevalence in the random sample of healthy pregnant women appears at first glance to be extremely high (6.7%).
IgM prevalence can be described by the following model:

$$IgM^{+} = m * p * N$$

where IgM^{+} is the number of women with IgM antibodies, m is the duration of the persistence of these antibodies, p is the risk of infection per month and N is the number of women in the study population. The data on IgM as a parameter that distinguishes between latent and recent infections are drawn from the part of the study population that consists of previously infected persons. The chance of a very recent infection which has not yet led to a measurable antibody production is very small and the presence of IgG antibodies is a good indication that infection once occurred. Thus N is the number of women with IgG antibodies at first examination.

Persistence during a limited period is essential if IgM is to be a useful parameter for retrospective differentiation between recent and latent infections. From this point of view a persistence of two months would be acceptable (m = 2). The risk of infection in the Netherlands has been estimated to be 12 per 1000 pregnancies (thus in 9 months), i.e. a monthly risk of 0.0013 (p = 0.001).[102] IgG antibodies were present in 849 of the first samples (N = 849). Introduction of these values into the equation yields 1.7 (95% confidence interval 0-4) IgM-positive samples. Obviously, the observed prevalence for the study group (IgM^+ = 129) differs markedly from this estimate; the number of IgM-positive samples found was 64 times higher. A higher IgM prevalence could be explained by a higher value of p or m. From the prospective study of pregnant women it appeared that the force of infection was much less than expected (see chapters 4 and 6). Observation during the first months of pregnancy was limited in this study since the first antenatal visit of the majority of women was not before the 12th week of gestation. It is generally accepted that the risk of primary infection is constant during the successive months of pregnancy. However, if primary infections were relatively more frequent at the beginning of pregnancy, this could this have contributed to the increased number of IgM-positive samples. Another explanation could be longer persistence of IgM. By applying the model the other way around, the IgM persistence (m) can be calculated; after introduction of the value 109 for IgM^+, i.e. the number of IgM-positive samples in the present investigation, it follows that m=128 months (95% confidence interval 104-152) or 10.7 years. This seems very unrealistic and therefore other factors must be involved.

It can be shown that the method used for the detection of IgM antibodies has a high specificity. As explained before, the absence of IgG is a good indicator of the absence of infection and thus can be used as a standard to evaluate IgM as a parameter for infection. The specificity of the IgM test can be estimated from the data of the present investigation. Comparing the IgM results with the IgG results a specificity of 1067/1087 = 0.98 (standard error 0.004) is derived. It is not possible to make an analogous analysis of the sensitivity, since the presence of IgG only indicates infection in the (far) past but gives no indication at all of the time lapsed since infection occurred.

The group of IgM-positive women might consist of women

- who really have been infected recently,
- with IgM antibodies that have persisted for more than two months who in fact have a latent infection,

- who have a positive test result due to measurement error,
- who have "natural antibodies", which may cause a positive IgM test that is not indicative of a history of toxoplasma infection.[126-128]

Assuming that there is no measurement error, the model used until now can be expanded to include a factor q representing other, unknown factors that cause a positive IgM antibody test (for example "natural antibodies"); moreover the specificity can be introduced into the model:

$$IgM^{+} = p * q * m * N / s$$

Table 7.3 shows, for varying values of m and p, which fraction of IgM seropositivity cannot be explained by m and p alone and thus must be attributed to an additional factor q. As before IgM^{+}=109, N=849; s=0.98.

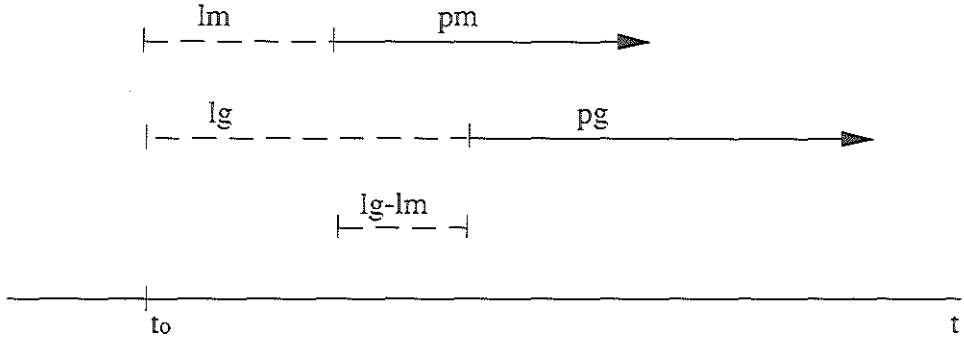
Table 7.3 Fraction of IgM-positive women in the population of previously infected women that cannot be attributed to a force of infection p and a persistence of IgM antibodies m (months).

m	p = 0.001	p = 0.005	p = 0.01
2	> 1.00	> 1.00	0.90
3	> 1.00	> 1.00	0.60
6	> 1.00	0.60	0.30
12	> 1.00	0.30	0.15
18	> 1.00	0.20	0.10
24	0.75	0.15	0.08
120	0.15	0.03	0.01

This latter model reveals that the prevalence of IgM in the group investigated is too high to be attributed merely to infection with *Toxoplasma gondii*, even if we assume prolonged persistence and a 10-fold higher force of infection. The prospective study indicated that it is not plausible at all to assume a higher force of infection for our country.
From the table it can be seen that when p=0.01 and m=6, 30% of the IgM prevalence must be attributed to other factors.

7.4.2 SPECIFIC IGM ANTIBODIES: INDICATOR OF ACUTE INFECTION.

The results of serodiagnosis of the 44 primarily infected women revealed that 13 of the samples preceding the sample that indicated IgG seroconversion were IgM-positive; this supports the classical interpretation of serology: IgM as the earliest sign of infection. But the investigation of samples from pregnant women without infection reveals that, within the context of screening, it is quite hazardous to interpret the IgM test in such a way: 64 of the 85 women with an IgM-positive sample exhibited no other sign of a possible primary toxoplasma infection: i.e. no IgG seroconversion.
The number of people for whom IgM is an early sign of infection must be weighed against the number of people who would have been alarmed needlessly because the positive IgM test was not a forerunner of IgG conversion and thus did not indicate primary infection.
An attempt was made to quantify the profit to be gained from detecting IgM in addition to IgG antibodies in order to be informed sooner about a possible infection in a seronegative woman. The process is illustrated schematically in figure 7.2.



t	time
to	moment of infection
lm	time lag before IgM antibodies are measurable
lg	time lag before IgG antibodies are measurable
pm	persistence of IgM antibodies
pg	persistence of IgG antibodies

Figure 7.2 Production of specific antibodies after infection in the course of time

First we assume that IgM is produced before IgG and that IgM persists

at least until IgG production starts (lg-lm≥0 and pm≥lg-lm). Examination is done every i weeks, the interval between observations in a prospective study. On the basis of the presence of IgG a fraction (i-lg)/i of the number of infections that have occurred in a period i will be recognized, on the basis of the presence of IgM a fraction (i-lm)/i.

When IgM detection is omitted, the loss of efficiency in a preventive programme with screening every eight weeks (i=8) can be quantified as follows:

- lg-lm=0, i.e. IgM is not detectable earlier than IgG, no loss.
- lg-lm=1, i.e. IgM is detectable one week earlier than IgG, for example IgM after one week and IgG after 2 weeks or IgM after 2 weeks and IgG after 3 weeks, lg-lm/i=1/8; this means recognition of 12.5% of the infections acquired during the previous two months is postponed until the next observation and therapy delayed. This equals 6.25% of the number of infections per month.
- lg-lm=2, i.e. IgM is detectable two weeks earlier than IgG, for example IgM after one week and IgG after three weeks, or IgM after two weeks and IgG after four weeks, lg-lm/i=2/8; this leads to a delayed diagnosis of 25% of infections in a two-month period of observation, or 12.5% of the number of infections acquired during a month.
- lg-lm=3, i.e. IgG is produced no earlier than three weeks after IgM, then lg-lm/i=3/8. Diagnosis of 37.5% of infections in every two-month period is delayed or nearly 20% of the monthly number of infections.
- lg-lm=4, i.e. the unrealistic situation that IgG is not detectable until 4 weeks after IgM, then lg-lm/i=4/8; recognition of 25% of the number of infections acquired during one month is delayed.

If we assume that IgM is no longer detectable from the moment of IgG production, then a fraction pm/i of the number of infections in the preceding period of observation (i) will be recognized earlier by adding IgM as a screening parameter. Analogous to the above calculations, this means that when:

- pm=1, pm/i=1/8=12.5% of the infections in a two-month period or 6.25% of the number in one month.
- pm=2, pm/i=2/8=25% of the infections in a two-month period or 12.5% of the number in one month.

It is not realistic to assume that a persistence of more than 2 weeks would not overlap IgG production.

If the screening interval is decreased, then the additional value of IgM for screening will be greater; if the screening interval is increased, the advantage

of screening for IgM and IgG will be less. For example, if screening were monthly instead of every two months the profit of IgM would change by a factor 2; if it were every three months instead of every two, it would decrease by a factor 2/3.
The model described in this section substantiates the view that it is not the moment after infection that IgM or IgG becomes measurable that determines the value of additional screening for IgM, but the difference between the two.

What would have been the profit of combined screening for IgG and IgM versus IgG screening during the TIP study (i=8 weeks), assuming that IgM is detectable two weeks before IgG?
During the TIP study, 44 seroconversions were traced during a mean observation period of 6 months per woman or 14.6 for every two months of observation (i=8 weeks). Diagnosis of 25% of these 14.6 infections, i.e. 3.65 infections, would have been delayed every two months or about 11 infections in the observation period of 6 months. This estimate is in accordance with the number of infected women in the TIP study who indeed had an IgM-positive sample prior to the sample that indicated seroconversion.

7.5 DISCUSSION

Secondary prevention of congenital toxoplasma infection relies on serological tests in order to trace infections acquired during pregnancy.
Demonstration of antibody production that was absent at previous examinations proves infection. An arbitrary interval between repeated examinations was chosen in order to recognize infection without too much delay, thus improving the chance that preventive therapy would not be too late.

What can be done if pregnant women are tested for the first time after several months of pregnancy and antibodies are present? She might have been infected around the time of conception or early in pregnancy. In order to exclude this small potential risk one is inclined to carry out additional serodiagnostic tests among women without complaints or any sign of infections. In the present investigation the use of IgM antibodies as a parameter for a recent infection has been evaluated.
Investigation of the presence of IgM in IgG-positive pregnant women with a primary toxoplasma infection by means of ELISA proved that these IgM

antibodies persist too long (over 3 months in 65% of infected women) to be useful as a parameter for retrospective identification of recent infections. If a new sample is compared quantitatively with the IgM-positive sample an increase in antibody level may support the diagnosis of a recent infection. In any case, without a previous seronegative blood sample, the risk of recent infection can never be excluded or confirmed definitely. Moreover, even if the diagnosis subsequently appears to be unlikely, anxiety is not easily relieved. Therefore it seems highly questionable whether it is acceptable to screen women in early pregnancy with the currently available diagnostic techniques for the detection of specific IgM. The majority of women will suffer because of the needless alarm.[201] A serological screening programme, unfortunately, is hampered by the fact that usually the woman does not begin prenatal supervision until a few months of pregnancy have already passed and thus some infections must be missed; to overcome this drawback it would be worthwhile to urge realization of the first examination as soon as pregnancy is confirmed or even before.

Antibodies of the IgM class are produced before antibodies of the IgG class. In accordance with this classical concept, infected women frequently (13/44) exhibited IgM before IgG. This shows that IgM detection is certainly important when diagnosing a toxoplasma infection in individual patients. A model was described to estimate the advantages of combined screening for IgG and IgM antibodies. If it is assumed that IgM becomes detectable two weeks before IgG and the women are tested every eight weeks, then it can be expected that during the TIP study 11 of the primary infections would have been traced earlier; this is very consistent with the 13 cases in which IgM antibodies were detected in the sample taken prior to seroconversion. However, prospective observation of successive blood samples from IgM-positive pregnant women showed that in 64 of the 85 cases IgM was measurable for a very long time (estimated median value 19 weeks) without subsequent IgG seroconversion.
That IgM seropositivity is not always indicative of an acute or recent toxoplasma infection, even if factors known to cause false-positive results are excluded, is also proven by the overall prevalence of IgM in sera from healthy women: the level was so high that it cannot be explained merely by a realistic force of infection, even if prolonged persistence is taken into account. Even with a highly sensitive and highly specific test, screening for a disease with a low incidence will be characterized by a high false-positive rate.[56]

Some people would benefit from screening for both IgG and IgM antibodies, but we have calculated that the profit (which is dependent on the frequency of repeated screening) is not very high, especially under the circumstances of a very low force of infection. Doubling the frequency may double the profit of additional screening for IgM; the relevance of this has to be judged in a cost-benefit analysis.

7.6 CONCLUSIONS

From the above-mentioned considerations it is concluded that

- the prevalence of specific IgM in an unselected population of pregnant women is much higher than could be assumed from a limited persistence of these antibodies after the moment of infection and the force of infection in our country;
- the use of IgM as a screening parameter frequently leads to further serodiagnostic examinations which may not always result in a definite diagnosis; as a consequence the mother's anxiety cannot be relieved adequately;
- the use of IgM as a screening parameter will raise the costs of screening from an economical as well as from a psychological point of view;
- screening for specific IgM antibodies among IgG-positive pregnant women without any sign of recent infection leads to an overestimation of the number of infections acquired during pregnancy;
- the profit of the detection of IgM antibodies in a prospective study based on serological screening for IgG every two months is very limited.
- It is not admissible to introduce additional IgM antibody assessment in routine screening.

8 GENERAL DISCUSSION AND CONCLUSIONS

8.1 INTRODUCTION

Since no effective therapy is available to prevent manifestations of congenital toxoplasmosis, prevention is the strategy par excellence to control congenital toxoplasmosis. The World Health Organization in Europe recommended that all countries consider the possibilities of a preventive programme; the programmes in France and Austria were cited as examples of successful disease control. At about the same time the Health Council in the Netherlands recommended a trial study. In the study described here both primary and secondary prevention were applied: repeated serological control of initially seronegative women allowed assessment of the incidence of primary infections during pregnancy when primary preventive measures were emphasized. In this chapter different strategies to prevent congenital toxoplasmosis in the Netherlands will be discussed.

8.2 POSSIBILITIES OF PRIMARY PREVENTION

The life cycle of *Toxoplasma gondii* itself indicates areas of intervention. It was Frenkel who stressed the possibilities of primary preventive measures based on this concept; he formulated the following directives:[58]

- Feed your cat only dried, canned or cooked meat.
- Keep your cat from foraging.
- Change litter boxes daily; disinfect them with boiling water.
- If pregnant, wear plastic gloves or delegate care of the cat to someone else.
- Use gloves when working in soil contaminated with cat faeces.
- Cover children's sandboxes when not in use.
- Watch for stray cats.
- Combat flies and cockroaches.

- Avoid eating raw meat; heat all meat thoroughly until it changes colour.
- Wash your hands before meals and before touching the face.

Avoid direct and indirect contact with cat faeces that may contain infectious oocysts, do not consume insufficiently cooked meat that may contain tissue cysts: that is what it is all about.

Next to the problem of the extent of preventive measures, there is the question of who should follow these measures: all women of child-bearing age, all women hoping to become pregnant soon, all women who are pregnant or all pregnant women known not to have been infected previously? It is clear that it is worthwhile to include information on the prevention of toxoplasmosis in general educational programmes on a healthy life style for future pregnant women. At the very least the mechanism of toxoplasma infection should be explained to a woman as soon as it is known that she is pregnant and she has to be informed about the need to follow preventive measures. It may be considered sufficient to perform one serological test in order to assess whether she is at risk for infection which would have the additional advantage that she will be more easily motivated to comply.[202] It is conceivable that this will result in excessive sensitivity and – since it cannot be guaranteed that infection is definitely excluded by the preventive measures – in feelings of guilt in the event of infection. On the other hand it may be asked whether it is acceptable to trouble someone who is immune already with needless preventive measures. In fact it is always worthwhile to accept the – not particularly restrictive – prohibition of raw or undercooked meat in order to prevent the transmission of other infectious diseases, such as salmonellosis and taeniasis. Moreover, it would be a more efficient use of resources to extend health education to all pregnant women since this would also offer protection to those with a false-positive screening test.[203]

The effect of primary preventive measures has never been assessed definitively. An investigation of changes in behaviour attributable to a 10-minute teaching session in prenatal classes on the prevention of congenital toxoplasmosis supports the effectiveness of primary prevention.[204]

A prospective study in Belgium showed a 34% reduction in the incidence of infection when primary prevention was advocated compared to the period when it was not. But when the study was continued the incidence rose again.[157,205]

It was suggested that the initial decrease could have been the result of a lower force of infection and not prevention.

No comparative data are available for our country. As mentioned in chapter

6 estimates of the force of infection from earlier cross-sectional studies are questionable. Our prospective study shows that only a limited number of infections occur when primary prevention is advocated, although it has not been proven that this is purely the effect of primary prevention.
Furthermore a relatively high percentage of primary infections occurred late in pregnancy: apparently primary prevention was not sustained up to the end of pregnancy. It is still a common fallacy that the foetus is only threatened by infectious diseases in the first three months of pregnancy. Thus it must be stressed that the risk of a toxoplasma infection for the unborn child still exists in the last trimester and pregnant women have to be reminded again and again of the need for preventive measures.

8.3 POSSIBILITIES OF SECONDARY PREVENTION

The aim of secondary prevention is immediate treatment of all primary infections that occur during pregnancy and thus to reduce the risk of transmission of infection to the unborn child.
How can primary infections be recognized? Serological techniques form the basis for diagnosis since there are hardly ever complaints or clinical signs indicating infection. During the TIP study none of the primarily infected women exhibited clinical signs. An infection can be diagnosed only by the appearance of specific antibodies in women who initially lacked these antibodies: a seronegative person becomes seropositive. Point of departure is a seronegative population that is repeatedly screened in order to detect seroconversion.
A limitation of this approach is that infections occurring around conception or early in pregnancy cannot be detected if a woman begins medical supervision after some months of pregnancy. There are no reliable techniques for the retrospective diagnosis of recent infections in healthy pregnant women. A high level of specific antibodies is not a useful indicator of recent infection;[82] the same applies for the presence of antibodies of the IgM class as discussed extensively in chapter 7. Misuse of these criteria results in an overestimation of the number of infections during pregnancy. Moreover it is ethically unacceptable in view of the psychological burden imposed on the woman, who may remain anxious throughout pregnancy and even until her child becomes one year old when the possibility of congenital infection can definitely be excluded. There is almost general agreement that treatment of women is indicated if it has been proven that infection was acquired

during pregnancy; this indication for therapy should not be extended to include "probable" infections. The report of the European meeting on the prevention of congenital toxoplasmosis held in Graz, Austria, 5-6 December, 1984, states the following on early infection: "Doubts have been expressed as to the value of elaborate serological tests on women who give a positive reaction when tested early in pregnancy. Acute infection already present during the first weeks of pregnancy accounts for less than 10% of cases of prenatal toxoplasmosis and the best diagnosis and treatment will not significantly reduce its amount. Moreover, infection at this time is likely to lead to abortion or to the birth of severely damaged babies, the desirability of whose survival is doubtful." Another limitation of secondary prevention is that the infection is always diagnosed some time after onset: there is an arbitrarily chosen interval between two consecutive examinations, antibody response must first reach detectable levels and there is the inevitable diagnostic delay.[206] In order to ensure the optimum possibility of recognizing infections during pregnancy - and recognizing them in time, one could:

- assess the immunity of women who intend to become pregnant in advance.
- begin serological testing as soon as it is known that a women has become pregnant.
- repeat serological testing regularly during pregnancy, for example every ten weeks.
- plan the last sample taken during pregnancy four weeks before the delivery date to allow for treatment before delivery in case a late infection has occurred.
- take a final sample at the postnatal check-up to exclude an infection at the very end of gestation.

As far as secondary prevention is concerned, it must be realized that infections assessed after birth do not contribute to the benefits of screening; the moment of intervention has already passed, the risk of transmission of the infection from mother to foetus cannot be influenced any more. The only purpose would be the identification of babies who were probably exposed prenatally to *Toxoplasma gondii*; it is very debatable whether medicine has anything to offer them.

Screening for primary toxoplasma infection during the TIP study was deliberately confined to the criterion of seroconversion. It was accepted that in doing so some recent infections occurring early in pregnancy that might have benefited from therapy were missed. On the other hand healthy women will be spared needless anxiety caused by a method with poor reliability.

However, even within these consciously chosen restrictions, there were still several obscure results in addition to the limited number of clear-cut seroconversions. It is hypothesized that exacerbation of an old latent infection with an antibody level below the detection level might simulate a primary infection. During the TIP study nine children of ten women presumed to have an old latent infection could be followed up to the age of one year: none of them was congenitally infected. Repeated serological screening of about 15,000 seronegative women revealed 44 primary infections. Twelve of these infections were recognized too late for timely intervention: pregnancy had already come to an end. So the ultimate yield of screening for the intended purpose of intervention was only 32 infections. Moreover, without intervention less than half of these infections would have resulted in congenital infection; intervention is expected to reduce the risk of congenital infection in the event of maternal infection by 50%, i.e. from 50% to 25%. Therefore the number of congenital infections prevented during the study might have been only eight.

In contrast to this number is the large number of women who were upset by an incorrect diagnosis that had to be revoked because sample exchange had occurred. It is generally accepted that administrative errors will be about 10%. Our study was highly automated in order to reduce the source of error at successive stages. Nevertheless errors cannot be completely excluded. Since screening for toxoplasma infections requires repeated examinations, instead of one single test, the number of errors will increase. In addition to comparing the number of errors with the number of actions, from the standpoint of quality control, the frequency of errors has to be compared with the frequency of the target event. The 23 sampling errors found in our study are of course only a fraction of the number that actually occurred, which is presumed to be at least twice as high (adding the counterpart of the sample with which it must have been exchanged). A number of exchanges was not recognized, fortunately without serious consequences since they involved exchange of two seropositive or two seronegative samples. It is not surprising that the number of errors, although extremely low (<0.1%), exceeded the number of primary infections in our study. Even if the number of errors had been ten times greater, this would not have been high compared to the number of samples, i.e. more than 70,000, although it would be disastrous if compared to the number of infections diagnosed.

8.4 INTERPRETATION OF THE NUMBER OF INFECTIONS DIAGNOSED DURING THE TIP STUDY

How can the low number of infections diagnosed during the preventive study in the Netherlands be explained?
Various possibilities can be postulated:
1. incorrect estimate of the risk of infection during pregnancy
2. decreased force of infection
3. decreased risk of infection due to the effect of primary preventive measures
4. decreased risk of infection due to the effect of primary preventive measures plus secondary prevention
5. underdetection of infections.

1. INCORRECT ESTIMATE OF THE RISK OF INFECTION DURING PREGNANCY

The number of children born with a congenital toxoplasma infection was estimated to be about 800 per year in the Netherlands.[102] This estimate was in fact derived indirectly from a study carried out in 1972-1976 of 1661 individuals.[71] The fraction of subjects with specific antibodies as a sign of prior infection was assessed for successive age groups in a cross-sectional study. Seroprevalence increased with age; from longitudinal interpretation of these data the risk of acquiring infection at different ages was estimated. From the viewpoint of congenital toxoplasma infections, the risk of a primary infection for pregnant women in our country was estimated to be 12.5 per 1,000 pregnant women or 2,125 cases when the birth rate is 170,000 per year.
We applied the same mathematical model to the results of several seroprevalence studies carried out in the Netherlands. For the purpose of comparison, the same age distribution of pregnant women (i.e. the 1975 age distribution) and the same birth rate (170,000 per year) were used in each analysis. Surprisingly analysis of these data sets resulted in quite different estimates, varying from 1095 to 2312 primary infections during pregnancy (see chapter 7). The estimate of 1095 was derived from the seroprevalence found for women participating in the TIP study, and thus might be assumed to be the closest approximation because of the characteristics of the sample: a large number of pregnant women.
The diverging results of mathematical analysis of the data of several seroprevalence studies presented in chapter 6 indicate that an accurate estimate of the risk of infection cannot be derived from such indirect investigations. Cohort effects probably influenced the age-specific seropre-

valence, which means that these data may not be interpreted longitudinally. The estimates in the report of the Health Council, that so far has served as a guideline for the planning of preventive studies, are now open to debate. The most reliable way to assess the risk of infection is a prospective investigation, preferably of the actual risk group: seronegative pregnant women. In 1964 Koppe et al. carried out such a study of 3040 women, 1821 of whom were examined at least twice. The possibilities of primary prevention were not known at that time; the role of the cat had not yet even been discovered. There were 21 women (1.1%) who exhibited a change of titre in the Sabin Feldman test from negative or very low to ≥512 and 42 women (2.2%) with a SF titre 512 at first testing.[206,207] Apart from the Koppe study no other prospective studies were performed in the Netherlands before the TIP study was initiated.

2. DECREASED FORCE OF INFECTION

The force of infection may have changed since the study of van der Veen et al. was carried out. There have been changes in the behaviour of the population towards a high risk consumption pattern (raw and undercooked meat), on the one hand, and changes in the rate of infection of livestock that depend on breeding methods, on the other. Foulon et al. observed that the incidence of primary infection has risen again, after a 4-year period of decreased rates attributed to the use of preventive measures. There was no indication that efforts to apply primary prevention had decreased. In order to explain this phenomenon they suggest a changing degree of infectivity.[157, 205] Actually there is a need for continuous evaluation of the force of infection by means of epidemiological investigations of human as well as animal populations.

3. DECREASED RISK OF INFECTION DUE TO THE EFFECT OF PRIMARY PREVENTIVE MEASURES

During the TIP study considerable attention was focussed on the ways in which pregnant women could reduce the risk of infection by adaptation of their behaviour. They were thoroughly informed about the measures to be taken. It seems reasonable to assume that, due to compliance with the advice, fewer toxoplasma infections occurred.

4. DECREASED RISK OF INFECTION DUE TO THE EFFECT OF PRIMARY PREVENTIVE MEASURES PLUS SECONDARY PREVENTION

Moreover it must be realized that the effect of primary prevention during

the TIP study was enhanced by repeated blood sampling; obviously the midwife or doctor was also reminded, by the need to carry out repeated blood sampling, to draw attention to the need for preventive measures. It is unlikely that the effect of primary prevention achieved would have been reached without the impact of additional screening.

5. UNDERDETECTION OF INFECTIONS

There is no doubt that more than 44 primary infections occurred during pregnancy in the study population:

- infections early in pregnancy

Even though it was advised that the first blood sample be taken as soon as possible during pregnancy or even before, 50% of women were tested for the first time after a gestation of more than twelve weeks. Thus three months of pregnancy may have passed before the women were informed about necessary preventive measures. Moreover infection acquired during these months was not diagnosed. In the future the effectivity of prevention can be improved by earlier serological screening and by incorporating information on toxoplasma infection prevention in expanded health educational programmes for women at childbearing age.

- infections late in pregnancy

On the other hand some infections late in pregnancy may also have been missed; the last blood sample was taken at birth and infections acquired during the last weeks of pregnancy may not yet have led to a measurable amount of antibodies at birth. The moment to start therapy of the mother in order to prevent transmission of parasites to the unborn baby has then passed. For this reason it may be worthwhile to consider taking the last blood sample from the mother at the postpartum check-up 6 weeks after birth, in order to obtain more complete information about the number of infections in a cohort of pregnant women.

- drop-out

One of the main problems of prospective investigations is the dropout rate, i.e. the number of women who are not followed to the end of pregnancy. We tried to keep this number to a minimum: the result of every test was sent to the midwife or physician together with the exact date when a new blood sample was expected from that particular woman. If a repeat sample was not received one month after the recommended date, a reminder was sent automatically together with a new, nearly completed, form (only the actual date of blood sampling had to be registered). Unfortunately there was not a standard reminder for the situation in which a baby was expected

and no sample (cord blood or postpartum maternal blood) was received. Ultimately there was a 30% dropout rate, consisting of pregnancies terminated by (spontaneous) abortions and women who no longer wished to participate.

8.5 CRITERIA FOR SCREENING

Back in 1968 Wilson and Jungner formulated some criteria for screening in a report on the principles and practice of screening for disease.[209]
Their criteria will be discussed here as they relate to a serological screening programme aimed at detection of toxoplasma infections during pregnancy that occur despite systematic primary prevention.

– *"The condition sought should be an important health problem."*

The classical triad of congenital toxoplasmosis, hydrocephaly, intracerebral calcifications and chorioretinitis, is rarely seen. If prenatal infection with *Toxoplasma gondii* does occur, there are usually no signs at all at birth. Although parasites are present (as bradyzoites within tissue cysts) infection can remain subclinical, even for years.
Reactivation of such a latent infection, however, leads to dissemination of active parasites (tachyzoites), causing tissue necrosis and decay. In vulnerable tissues, such as the nervous system and the retina, this may result in loss of function. The arousal of a latent congenital infection may occur at any moment, causing ophthalmological defects as late manifestations even after more than a decade. In particular the progressive loss of vision, possibly resulting in blindness, seems to be a potential risk for all congenitally infected children. That is why congenital toxoplasmosis is considered a relevant health problem: the impact of congenital toxoplasmosis surpasses by far morbidity in the neonatal period and becomes evident even much later.
From our prospective study it is estimated that in the Netherlands 400 primary infections take place during pregnancy every year notwithstanding proper education; if an average transmission rate of 40% is assumed (in absence of secondary prevention), then 160 children with a congenital toxoplasma infection will be born every year.

– *"The natural history of the condition, including development from latent to declared disease, should be adequately understood."*

Long-term follow-up studies have consistently illustrated the inevitable evolution of an asymptomatic subclinical congenital toxoplasma infection to clinically manifest, predominantly ocular, toxoplasmosis with progressive, generally unilateral visual impairment. From the study of Koppe[86] it can be derived that 25% of these children will suffer from severe impairment of vision in one eye before the age of 20 years; in the case of 160 congenital infections per year this results in 40 cases of severe ocular toxoplasmosis. The risk of neurological and developmental defects in initially asymptomatic children is less evident (see chapter 1).

– *"There should be an accepted treatment for patients with recognized disease."*

With respect to the screening of pregnant women this criterion has to be applied to the treatment of pregnant women which is in fact prescribed as prophylaxis for the foetus. Without treatment the rate of transmission of infection to the foetus is below 50%. Moreover it is not certain that foetal infection will not occur; therapy is thought to be effective in at least 50% of cases. This means that a considerable percentage of infected women are treated without success. Since there is no way to predict who will profit and there are no other possible interventions, treatment of proven maternal infection during pregnancy is thought to be acceptable.
There is no agreement on the effectivity of prophylactic treatment of asymptomatic children after birth in order to prevent later clinical manifestations, since the drugs only affect active proliferating parasites and not the slumbering stages characteristic of latent infection. (Even in case of overt congenital disease, which is often the result of active infection some months earlier during foetal life, postnatal treatment may have no effect since infection has already become latent.)

– *"There should be a recognizable latent or early symptomatic stage."*

It is possible by means of one serological assay to distinguish those who were infected from those who were not and thus are at risk, but it is not possible to assess in an accurate way whether the infection occurred recently or long ago. Therefore infections acquired early in pregnancy before a first sample was taken (probably about 30%), cannot be traced adequately. Primary infections occurring after the first blood test can be traced by repeated examination at regular intervals of women who have never been infected. Thus not all toxoplasma infections occurring during pregnancy can be identified by means of systematic screening of pregnant women.

Since several examinations are required, screening for toxoplasma differs from other screening programmes (for example, phenylketonuria and congenital hypothyroidism are assessed in a single examination of neonate serum). Needless to say, it does not make any sense to screen newborn babies for congenital toxoplasma infections since there is neither a useful parameter for the recognition of congenital infection at birth nor effective prophylaxis against later morbidity due to the subclinical infection.

– *"There should be a suitable test or examination."*

Semi-quantitative serological tests are useful in large-scale population studies. However, the use in screening programmes of serological techniques that were originally developed for diagnostic purposes is risky and demands careful interpretation. In the event of seroconversion the test results must always be confirmed by means of simultaneous testing of a pair of consecutive samples. For a national prevention programme involving repeated serological tests, it is imperative that test results from different laboratories all over the country be comparable: continuous inter-laboratory quality control is a prerequisite for a programme relying on repeated examinations.
No techniques are available to determine soon after delivery whether a newborn baby has congenital toxoplasma infection.

– *"Facilities for diagnosis and treatment should be available."*

Facilities are available. Screening can be performed efficiently due to extensive automation of laboratory procedures. Infected women and children can be treated by obstetricians or paediatricians in the Netherlands. However, the physicians' knowledge of *Toxoplasma gondii* must be updated to guarantee adequate counselling of patients.[210]

– *"The test should be acceptable to the population."*

A blood sample is obtained from all pregnant women at the first prenatal visit; only repeated testing leads to an extra load for the woman who will cooperate after an explanation. As far as we know only a few women withdrew from the TIP study because of repeated blood sampling.
More difficulties were encountered with prescribed therapy in the case of infection. Campaigns against the use of drugs during pregnancy have been successful as indicated by the psychological resistance to pharmacotherapy.

Repeated examinations for at least one year of children that appear perfectly normal at birth is not easily accepted.

- *"There should be an agreed policy on whom to treat as patient."*

There is consensus that all pregnant women with proven primary infection during pregnancy, as indicated by a seroconversion, have to be treated. No agreement, however, exists on treatment of women with an infection early in pregnancy which has not been proven but is only suspected on the basis of a serological test (e.g. the presence of IgM or a high IgG antibody titre, neither of which is sufficient proof).

- *"The cost of case-finding (including diagnosis and treatment of patients diagnosed) should be economically balanced in relation to possible expenditure on medical care as a whole."*

Using estimates of the number of infections occurring during pregnancy, the Dutch Health Council calculated that serological screening would not be cost effective in our country. Now that the results of the prospective study have revealed that the number of infections to be traced is even smaller, it is even more questionable whether the additional costs of repeated blood tests for half of the pregnant population are justifiable as an extra prevention measure after introduction of systematic primary prevention.

8.6 INTERNATIONAL DEBATE

In spite of the examples of France and Austria, the introduction of screening of pregnant women for toxoplasmosis has not progressed. There is continuous debate in international literature on the desirability of primary and/or secondary prevention.
In the meanwhile serological screening is being performed haphazardly without prior assessment of conditions for quality control. That is why trial studies have been advocated, e.g. in the USA,[211] the Netherlands,[102] and the United Kingdom.[212]
The proponents and opponents contrast in the emphasis they place on the effect of screening on individual persons versus the effect on the population. The effect for an individual woman who acquires a primary infection is that infection of her child may be prevented. What the target group does

not know is that infection during pregnancy does not always result in infection of the unborn child (the risk of transmission averages 50%) and that the proposed intervention does not guarantee that infection will not be transmitted (probable reduction of the risk by 50% yields a 25% transmission rate). Moreover often the women are not told that only a certain percentage of the infections occurring during pregnancy are traced.
Erroneously it is suggested that participation in a screening programme only entails advantages; the programme easily appears too rosy as in the first brochure on primary and secondary prevention (first edition, see appendix 1a) distributed to all women participating in the TIP study.
The effect for the population is that the incidence of congenital toxoplasma infections is reduced but that the psychological burden and community expenses will be high. The psychological costs, particularly for those who receive incorrect positive results, are often disregarded.[213]
Several authors have warned that a high frequency of false-positive results is to be expected when the incidence is low. If a test with both a sensitivity and a specificity of 99% is used, the predictive value of a positive test in the case of a seroconversion rate of 10 per 1000 (situation in France) would be 92%; in contrast, for rates between 2 per 1000 and 6 per 1000 (USA) it would range from 18 to 37%.[214] For each real case of toxoplasmosis many pregnant women would be falsely identified as having the disease. Similarly the estimate of false-positive results to be expected in the United Kingdom (seroconversion rate 2 per 1000[215]) is 6000 versus 1200 maternal infections.[56] Opposite the number of false-positive findings is the number of infections not recognized in time due to inevitable limitations of the programme (for example, early and late infections). It is unrealistic to claim the maximum result as the benefit of the programme since that will not be reached. In our study 12 of the 44 primary infections were not recognized before birth when the moment for effective intervention had already passed.
The generation of investigators who initiated the preventive programme in France seems to be reluctant to consider its side effects.[158] But recently there was a remarkable report from some investigators who criticize the current strategy in their country and argue for primary prevention by means of health education.[216] In contrast to earlier estimates of seroprevalence (90%),[34] seroprevalence is now thought to be 72% for pregnant women of French origin and 51% for migrant women.[217] As a consequence more women must participate in the screening programme which in turn leads to higher costs.
The results of a prospective study of life style characteristics provided support

for primary prevention. In addition to eating undercooked meat and owning a cat, eating regularly in restaurants was identified as a risk factor.[218]
Limitation of such a programme to primary prevention has been advocated in recent literature,[15] with a plea by some to assess immunity before conception[219] or at the first antenatal visit.[201] Secondary prevention is favoured by others who propose to combine toxoplasma screening with screening for rubella and cytomegalovirus, thus reducing the costs.[220,221]
Without accurate data on incidence, prevalence and transmission rates, on the one hand, and data on the effectiveness of a screening programme, on the other, a reliable cost-benefit analysis is difficult to make. Many authors keep harping on the lack of epidemiological data on prevalence and incidence,[222,223] and this also applies in the Dutch situation. While some authors are convinced that spiramycin therapy decreases the rate of transmission of parasites,[34] others emphasize the lack of proof of the effectivity of the therapy during pregnancy;[214,216] however some believe that evaluation by means of a randomized trial is impossible for ethical reasons, at least in France[216] and even in other countries.[224] Another opinion is that, from the ethical point of view, such a trial is in fact imperative when doubts about the effects of that "ethical" treatment arise since otherwise misplaced faith in spiramycin will continue to mislead doctors and patients.[225] In the present investigation the transmission rate for untreated women was high (9/11 or 82%) compared to the rate for treated women (3/32 or 9%), but all of these untreated women were infected during the third trimester when the risk of vertical transmission is very high.
There are not sufficient data to be able to draw conclusions about the effectivity of treatment during pregnancy.
Henderson et al., who made a sensitivity analysis of the cost-benefits, dealt with the lack of epidemiological data by taking a range of values which would include the true value of each estimate.[226] If the minimum estimate of benefit exceeds the maximum estimated cost, the programme would be deemed worthwhile. It seemed in 1984 that for the United Kingdom a health educational programme would be more likely to save resources than a screening service. Neither the costs of false-positive and false-negative results nor the psychological costs were included in their analysis. A cost-benefit analysis in Norway based on epidemiological data from studies carried out in the late seventies/early eighties gave a ratio of >13:1 for primary prevention and a ratio of 3:1 for screening with an SF test. Only one serological repeat test for seronegatives (87% in Norway) is included.[227]
No cost-benefit studies have been published on the "established health

policies" in France and Austria; they appear to have been motivated by humanitarian and political considerations.[228] Evaluation of these screening programmes which has never been performed should include both epidemiological and psychological aspects of the programme.[201] As mentioned before, the cost-benefit analysis for the Netherlands performed by the Health Council in 1983 did not show that serological screening could be cost-effective.[102]

8.7 CONCLUSIONS

Our prospective study showed that only a limited number of primary infections can be traced by serological screening. As we knew in advance, it is impossible to decide whether this should be attributed to primary prevention or whether it is the result of a low endemicity. The study indicates that the extent of the problem of congenital toxoplasmosis in the Netherlands – when primary prevention is applied – does not justify the addition of secondary prevention. The latter is not as straightforward as always suggested, and it fails to live up to expectations since some infections will not be traced and there are a lot of side-effects.
Enriched by the experience of our large-scale study on both primary and secondary preventive measures, we now favour the original approach of the Dutch Health Council: control of congenital toxoplasmosis by primary prevention.
As far as primary prevention is concerned, it is not sufficient to mention casually the potential risk of undercooked meat and cat faeces or to put a number of information leaflets next to the magazines in the waiting room. The message has to be explained to the women by those who are responsible for prenatal care. In addition to nationwide distribution of the information brochure, other approaches must also be used: toxoplasma infection prevention has to be included in general campaigns for a healthy life style for (future) pregnant women and visual material should be available at prenatal classes.

The decision to discourage secondary prevention requiring serological screening and to rely on primary preventive measures implies that further investigations will have to be carried out to monitor the epidemiology of toxoplasmosis: i.e. the prevalence of infections in humans, especially the incidence in pregnant women, and the prevalence of infections in livestock.

It must be kept in mind that this strategy, i.e. only primary prevention, could be dropped if it becomes apparent that the effect of health education is not being achieved with a standard programme (lacking the special attention of a trial study), the force of infection increases, new serological techniques become available (for example, "bedside" tests that would eliminate many logistical problems or simple tests that recognize recent infections retrospectively), new therapeutics with a higher effectivity are discovered or the efficacy of screening can be increased by the introduction of a tool for tracing early as well as late infections during pregnancy.

In summary:
- Only a proportion of the primary infections occurring during pregnancy (approximately 65%) can be traced, due to the inevitable limitations of a serological screening programme.
- There were 44 (0.16%) infections in a prospectively followed cohort of 27.967 pregnant women who were thoroughly informed about preventive measures.
- Infections that are not recognized before the end of pregnancy cannot benefit from therapeutic intervention: 12 out of the 44 in the TIP study.
- Therapeutic intervention is not able to prevent transmission of infection from mother to foetus in all cases. The overall transmission rate can be reduced by half from 50% to 25%.
- The parents of a child potentially at risk for congenital infection cannot be reassured, with the presently available techniques, until the child is one year old.
- Apart from the infections traced, there will be a considerable number of women who will suffer needless anxiety: those with an infection that later on appears to be a latent infection (10 in the TIP study) and the victims of sampling errors (23 in the TIP study).

Under current circumstances it is not justifiable to state that in the Netherlands healthy pregnant women in general would profit from a screening programme. Compared to the limited number of infections traced, there is the large number of women who will suffer needless anxiety during pregnancy. Moreover the anxiety frequently cannot be relieved definitely until the child has reached the age of one year.

SUMMARY

In this thesis the results of a large-scale study of preventive measures against toxoplasma infections in pregnant women are reported.

Literature on *Toxoplasma gondii,* toxoplasma infections and toxoplasmosis is discussed in chapter 1. Special attention is directed toward the epidemiological aspects as they relate to the possibilities of preventing congenital toxoplasmosis. The historical background is presented as an introduction to the actual study. The initial aim of separate evaluation of primary and secondary prevention had to be abandoned due to various circumstances. As a result, the study was finally designed to evaluate the simultaneous application of both primary and secondary preventive measures in order to be able to establish guidelines and prerequisites for a future national preventive programme.

In chapter 2, the IgM prevalence in a chort of newborns is described. The serological determinations were carried out using blood samples collected on filtre papers. Although it is known that not all newborns infected congenitally with *Toxoplasma gondii* produce specific IgM antibodies that can be considered indicative of infection, it was believed that the IgM prevalence in a chort of newborns could be used as an epidemiological parameter for evaluation of the effect of preventive measures introduced at a later stage. The IgM prevalence was 0.08% for 32,000 samples. This value was below the expected value (0.125%) which could indicate a lower incidence of congenital toxoplasma infections in the Netherlands. Further prospective studies could provide the answer to this question. In view of the low level of the IgM prevalence, the validity of this parameter for evaluation of prevention came under discussion; to obtain a reliable evaluation of the effect of prevention the size of the study population would have to be increased to such an extent that this was not considered feasible. Therefore, it was decided that determination of the IgM prevalence among newborns would not be continued.

The design and organization of the preventive study are described in chapter 3. At their first pregnancy check-up, the pregnant women were informed about the study and about the measures that they should take in order to prevent a toxoplasma infection during pregnancy. In addition a blood sample was taken for determination of their immune status. Women without serological indications of a previous infection were requested to provide blood samples for renewed screening in the 18th, 24th and 32nd week of pregnancy; the last sample was a cord blood sample drawn after birth of the child. The participation of physicians and midwives as well as their patients is analysed. Between March 1987 and March 1988 more than 28,000 women were enrolled in the study.

The results of the serological screening are presented in chapter 4. The mean seroprevalence (IgG) was 45.5%; this prevalence increased with age from 38.2% for 15-19 year-olds to 65.8% for 40-44 year-olds. Repeated serological screening of seronegative women (mean period of observation: 6 months) yielded 244 cases of suspected intercurrent toxoplasma infection.
Seroconversion, and therefore primary infection, could be confirmed in 77 cases after simultaneous analysis of two successive blood samples. In the remaining cases there was merely a marginal shift in the antibody level around the established limits of the test. After repeated serological screening in the period after the infection was detected, however, the diagnosis had to be revoked 33 times: 23 cases involved sample errors while 10 women became seronegative again in the course of time. Further investigation led to the assumption that this latter group consisted of women with an old latent infection and an antibody level just below the limit that temporarily increased as a result of pregnancy. Several case histories are described.
Ultimately 44 primary infections were detected: 12 of these cases were however not detected until after delivery so that treatment of the mother to prevent transmission of the infection to her unborn child was not possible.

Only rarely do children with a congenital toxoplasma infection exhibit symptoms of their disease at birth; there is, however, a chance of later (especially ophthalmological) manifestations. For this reason, the diagnosis is usually established on the basis of the serological findings, which however are dominated in the early stages of the first year of life by the passively acquired antibodies of the mother. In order to demonstrate the production of antibodies by the child itself, which is a sign of congenital infection, repeated examination is necessary. A congenital infection can only be

excluded definitely if antibodies cannot be demonsrated at the end of the first year of life. All children of infected pregnant women were examined paediatrically, ophthalmologically and serologically at the ages of 1 month, 6 months and 1 year. In chapter 5 the findings of these examinations are presented. Follow-up of one of the 44 children was not completed; of the remaining 43, 12 were infected as demonstrated by chorioretinitis in 2 cases (diagnosed at 3 weeks and 7 months, respectively), intracerebral calcifications without further neurological abnormalities in 1 case and only serological indications of infection in 9 cases. In 9 cases the mother of the infected child was not treated because the infection was diagnosed too late. This would seem to indicate an effect of treatment but the data from this study do not permit conclusions because the numbers are too small and because all untreated infections developed in the last trimester when the risk of transmission is the greatest.
None of the children was treated postnatally because none exhibited signs of parasitic activity and the drugs available at present are not effective when the infection has become latent. We do not share the view of other investigators that prophylactic treatment is indicated for all infected children during the first year of life.

The estimates of the size of the problem of congenital toxoplasmosis in the Netherlands are based on analyses of age-specific seroprevalence. In chapter 6 such analyses are applied to the serological profiles obtained from various cross-sectional studies, including the seroprevalence established on the basis of the first examination of women who participated in the TIP study. Although the same mathematical method was always used, the estimates of the expected annual number of toxoplasma infecions among pregnant women varied widely: from 1095 to 2312, which illustrates the uncertainty of estimates obtained in this manner. The estimate used officially so far is 2125, whereas the seroprevalence measured during the TIP study was 1095. There are indications that cohort effects could have played a role here which would mean that the requirements for longitudinal interpretation of data from a cross-sectional study have not been satisfied. After correction for the limited period of observation, the number of infections diagnosed during this prospective study of pregnant women can be converted to the total annual number of infections to be expected in the Netherlands when primary prevention is applied: i.e. about 400.

During the TIP study, IgG antibodies were determined in order to establish

the presence of infection. In chapter 7 the value of the determination of IgM as an additonal parameter is considered. In a random sample of the blood samples collected for the TIP study IgM antibodies were determined. The prevalence was much higher than was to be expected on the grounds of the assumed force of infection and the limited persistence of these antibodies after infection. A mathematical model was used to show that IgM antibodies persist so long after infection that determination of IgM antibodies cannot be used to establish retrospectively that an infection may have recently occurred. In a prospective study, a toxoplasma infection will be recognized sooner in a number of cases on the basis of the appearance of IgM antibodies. In the case of regularly repeated screening, for instance every two months, the profit of IgM is however limited and women who in fact do not have an intercurrent infection will have to undergo frequent supplementary diagnostic examinations. The costs of screening will then also increase considerably. Moreover screening for IgM antibodies among pregnant women will cause extensive unnecessary anxiety that is not easily relieved.

In chapter 8 the possibilities and impossibilities of primary and secondary prevention are considered. If primary prevention is introduced, the number of infections detected in the Netherlands will be limited. With the serological techniques and the logistic means now available, it appears that it still will not be possible to detect all existing infections (on time). Infections early in pregnancy cannot be recognized unequivocally and precisely; infections late in pregnancy can be detected but often not early enough for initiation of therapy to prevent congenital infection.
The criteria of Wilson and Jungner for the screening of healthy individuals are applied to screening for the purpose of secondary prevention after a number of infections have already been prevented by primary prevention. It is clear that not all of the criteria are satisfied to a sufficient degree. International discussions on the feasibility of prevention are assessed.
On the basis of the results of the prospective preventive study, it is concluded that secondary prevention is not feasible in the Netherlands.
It is recommended that extensive educational programmes that focus on primary prevention of toxoplasma infections be organized for (future) pregnant women in the Netherlands. The effect of conscientious primary prevention will have to be established by means of a continuous epidemiological study of the incidence of toxoplasma infections among pregnant women.

SAMENVATTING

In dit proefschrift wordt verslag gedaan van de ervaring met toepassing van preventie van toxoplasma infecties bij zwangere vrouwen, opgedaan in een grootschalig proefonderzoek.

In hoofdstuk 1 wordt de literatuur met betrekking tot *Toxoplasma gondii*, toxoplasma infecties en toxoplasmose besproken. In het bzonder wordt aandacht geschonken aan de epidemiologische aspecten in verband met mogelijkheden tot preventie van congenitale toxoplasmose. Ter inleiding op het onderzoek wordt de voorgeschiedenis toegelicht. Door omstandigheden moest worden afgezien van afzonderlijke evaluatie van primaire en secundaire preventie. Het uiteindelijke onderzoek kreeg het karakter van een proefonderzoek met gelijktijdige toepassing van zowel primaire als secundaire preventie waaraan richtlijnen en voorwaarden voor een eventueel later te introduceren landelijk preventief programma zouden kunnen worden ontleend.

In hoofdstuk 2 wordt de anti-Toxoplasma IgM prevalentie in een cohort pasgeborenen gerapporteerd. De serologische bepaling werd verricht op bloedmonsters verzameld op filtreerpapier. Hoewel bekend is dat niet alle pasgeborenen die congenitaal met *Toxoplasma gondii* zijn geïnfecteerd specifieke IgM antistoffen produceren als teken van infectie, werd gemeend dat de IgM prevalentie in een cohort pasgeborenen bruikbaar zou zijn als epidemiologische parameter, aan de hand waarvan het effect van later toe te passen preventieve maatregelen zou kunnen worden geëvalueerd. De IgM prevalentie bedroeg 0.08% bij onderzoek van 32,000 monsters. Dit cijfer lag beneden de verwachte waarde (0.125) hetgeen zou kunnen wijzen op een lagere incidentie van congenitale toxoplasma infecties in Nederland. Nader prospectief onderzoek zou hierover uitsluitsel kunnen geven. Gezien het lage niveau van de IgM prevalentie kwam de bruikbaarheid van deze parameter voor evaluatie van preventie alsnog ter discussie; om een be-

trouwbare uitspraak te kunnen doen over het effect van preventie zou de omvang van de studie-populatie dermate moeten worden vergroot dat dit niet haalbaar werd geacht. Derhalve werd van een verdere bepaling van de IgM prevalentie bij pasgeborenen afgezien.

De opzet en organisatie van het preventieve onderzoek worden beschreven in hoofdstuk 3. Zodra de zwangere vrouwen onder verloskundige begeleiding kwamen, werden zij geïnformeerd over het onderzoek en over de maatregelen die zij zouden moeten nemen teneinde een toxoplasma infectie in hun zwangerschap te voorkomen. Voorts werd een bloedmonster afgenomen voor bepaling van de immuunstatus. Vrouwen zonder een serologische aanwijzing voor een eerder doorgemaakte infectie werden opnieuw onderzocht bij een zwangerschapsduur van 18, 24 en 32 weken; een laatste controle vond plaats in navelstrengbloed na de geboorte van haar kind. Overzichten van de participatie van artsen en verloskundigen en van hun patiënten worden gepresenteerd. Tussen maart 1987 en maart 1988 werden ruim 28.000 vrouwen voor het onderzoek ingeschreven.
De resultaten van serologische screening worden gerapporteerd in hoofdstuk 4. De gemiddelde seroprevalentie (IgG) bedroeg 45,8%; deze nam toe met de leeftijd van 38,2% bij 15- tot 19-jarigen tot 65,8% bij 40- tot 44-jarigen. Bij herhaalde serologische controle van de seronegatieve vrouwen (gemiddelde observatieduur 6 maanden) rees 244 maal verdenking op een intercurrente toxoplasma infectie. De seroconversie kon 77 maal worden bevestigd na simultaan onderzoek van twee opeenvolgende bloedmonsters, waarmee de primaire infectie was vastgesteld. In de overige gevallen was slechts sprake van een marginale verschuiving van de hoeveelheid antistoffen rond de grenswaarde van de test. Na herhaalde serologische controle in de loop van de tijd nadat de infectie was vastgesteld, moest de diagnose echter 33 maal worden herroepen: 23 maal was er een monsterverwisseling in het spel geweest, 10 maal werd geconstateerd dat de vrouw na verloop van tijd weer seronegatief werd. Op grond van nader onderzoek werd verondersteld dat deze laatste groep in feite vrouwen omvatte met een oude latente infectie en een beneden de grenswaarde gedaalde hoeveelheid antistoffen die onder invloed van de zwangerschap tijdelijk was gestegen. Enkele voorbeelden worden beschreven.
Uiteindelijk werden 44 primaire infecties geconstateerd, waarvan 12 pas bij onderzoek na de geboorte van het kind zodat behandeling van de moeder ter voorkoming van transmissie van de infectie naar haar ongeboren kind niet plaats heeft kunnen vinden.

Zelden vertonen kinderen met een congenitale toxoplasma infectie ziekteverschijnselen bij de geboorte; er is echter een kans op latere klinische manifestaties, met name op oogheelkundig gebied. Derhalve wordt de diagnose doorgaans gesteld op het serologische beeld, dat echter in het begin van het eerste levensjaar gedomineerd wordt door passief van de moeder verkregen antistoffen. Om antistofvorming door het kind zelf als teken van congenitale infectie te kunnen vaststellen is herhaald onderzoek noodzakelijk. Pas als er op het eind van het eerste levensjaar geen antistoffen meer meetbaar zijn, kan een congenitale infectie definitief uitgesloten worden geacht. In dit onderzoek werden alle kinderen van geïnfecteerde zwangeren paediatrisch, oogheelkundig en serologisch onderzocht in de eerste levensmaand, op de leeftijd van een half jaar en op de leeftijd van één jaar. In hoofdstuk 5 worden de bevindingen gerapporteerd. Bij één van de 44 kinderen werd het vervolgonderzoek kort na de geboorte afgebroken; van de overige 43 kinderen bleken er 12 geïnfecteerd, waarvan twee met een chorioretinitis (geconstateerd op de leeftijd van drie weken respectievelijk zeven maanden), één met intracerebrale calcificaties zonder verdere neurologische afwijkingen en negen met slechts serologische aanwijzingen voor infectie. Bij negen van de geïnfecteerde kinderen was de moeder niet behandeld omdat de infectie te laat werd geconstateerd. Dit lijkt suggestief voor een effect van behandeling, doch de gegevens uit dit onderzoek laten geen conclusie toe vanwege de kleine aantallen en aangezien alle niet behandelde infecties in het laatste trimester optraden waar het risico op transmissie het hoogst is.
Geen van de kinderen werd postnataal behandeld, aangezien er bij geen van hen tekenen van parasitaire activiteit waren; de beschikbare medicamenten hebben immers geen effect wanneer de infectie latent is geworden. De visie van andere onderzoekers dat prophylactische behandeling van alle geïnfecteerde kinderen gedurende het eerste levensjaar is geïndiceerd, wordt niet gedeeld.

De schattingen van de omvang van het probleem van congenitale toxoplasmose in Nederland zijn gebaseerd op analyses van de leeftijdsspecifieke seroprevalentie. In hoofdstuk 6 zijn dergelijke analyses toegepast op de serologische profielen die ontleend zijn aan verschillende transversale onderzoekingen, onder andere de seroprevalentie bij eerste onderzoek van de vrouwen die deelnamen aan het TIP onderzoek. Hoewel steeds dezelfde mathematische methode wordt toegepast, lopen de schattingen van het jaarlijks te verwachten aantal toxoplasma infecties onder zwangeren zeer uiteen: van 1095 tot 2312, waarmee de onzekerheid van op deze manier

verkregen schattingen is geïllustreerd. De tot op heden gehanteerde schatting bedroeg 2125, die op grond van de in het TIP onderzoek gemeten seroprevalentie 1095. Er zijn aanwijzingen dat cohort-effecten in het spel zijn, waardoor niet is voldaan aan de voorwaarden voor longitudinale interpretatie van gegevens uit een transversaal onderzoek. Corrigerend door de beperkte observatieduur, wordt het aantal bij prospectief onderzoek onder zwangere vrouwen geconstateerde infecties gegeneraliseerd naar een totaal jaarlijks in Nederland te verwachten aantal van omstreeks 400, wanneer primaire preventie wordt toegepast.

Tijdens het TIP onderzoek werden antistoffen van de IgG klasse bepaald ter opsporing van infecties. In hoofdstuk 7 komt de waarde van bepaling van IgM antistoffen bij zwqangeren als additionele parameter aan de orde. In een steekproef uit de tijdens het TIP onderzoek verzamelde monsters werd alsnog een IgM bepaling verricht. De prevalentie was veel hoger dan op grond van de verwachte infectiedruk en een beperkte persistentie van deze antistoffen na infectie verwacht mocht worden. Met een mathematisch model wordt aangetoond dat IgM antistoffen zo lang na infecties persisteren dat bepaling van IgM antistoffen niet bruikbaar is om retrospectief vast te stellen of de infectie mogelijk recent heeft plaats gevonden. Bij prospectief onderzoek zal een toxoplasma infectie in een aantal gevallen eerder herkend worden op geleide van de verschijning van IgM antistoffen. Bij regelmatig herhaalde screening, bijvoorbeeld om de twee maanden, is het rendement van IgM screening echter beperkt, terwijl frequent aanvullend diagnostisch onderzoek nodig zal blijken in gevallen waar toch geen sprake blijkt te zijn van een intercurrente infectie. De kosten van screening zullen daarmee eveneens aanzienlijk toenemen.
Bovendien zal screening op IgM antistoffen onder zwangeren veel overbodige onrust veroorzaken die niet eenvoudig is weg te nemen.

In hoofdstuk 8 wordt ingegaan op de mogelijkheden en onmogelijkheden van primaire respectievelijk secundaire preventie. Gegeven primaire preventie is het aantal opgespoorde infecties in Nederland beperkt. Met de thans beschikbare serologische technieken en logistieke mogelijkheden blijkt het niet mogelijk alle alsnog optredende infecties (op tijd) op te sporen.
Infecties vroeg in de zwangerschap kunnen niet eenduidig en trefzeker worden herkend, infecties laat in de zwangerschap kunnen worden opgespoord doch vaak niet zo dat tijdig therapie ter voorkoming van congenitale infectie kan worden ingesteld.

De criteria van Wilson en Jungner voor screening van gezonde personen worden toegepast op screening in het kader van secundaire preventie nadat door primaire preventie reeds een aantal infecties is voorkomen. Het is duidelijk dat niet in voldoende mate aan alle criteria is voldaan. De internationale discussie over de wenselijkheid van preventie wordt becommentarieerd.

Op grond van de resultaten van het prospectieve preventieve onderzoek wordt geconcludeerd dat het niet wenselijk is dat secundaire preventie in Nederland wordt toegepast. Aanbevolen wordt om uitgebreide voorlichtingscampagnes over de mogelijkheden van primaire preventie van toxoplasma infecties voor (toekomstige) zwangeren in Nederland te organiseren. Het effect van gedegen primaire preventie zal gelijktijdig in een doorlopend epidemiologisch onderzoek naar de incidentie van toxoplasma infecties onder zwangeren moeten worden bevestigd.

ADDENDUM: LOGISTICS AND AUTOMATION

1 INTRODUCTION

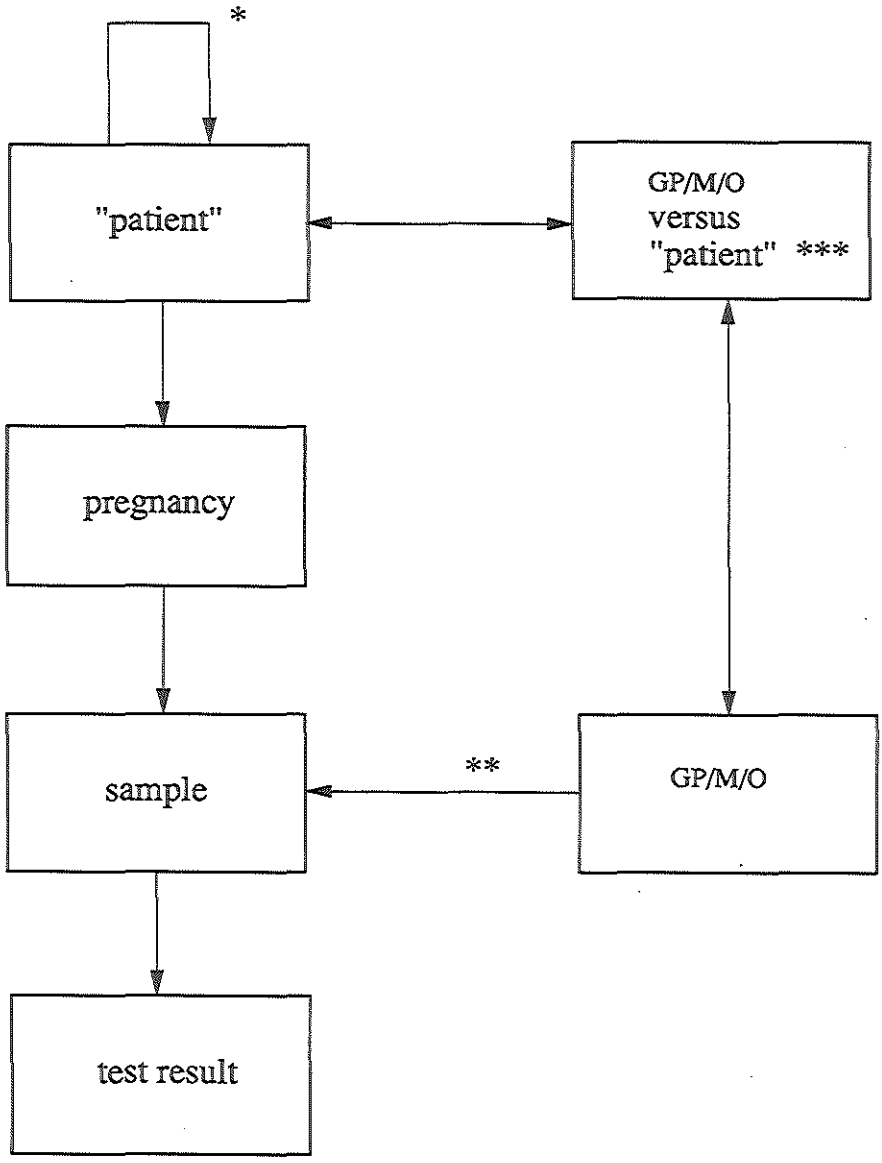
The TIP study was a large-scale study with a target population of 38,000 individuals who were expected to provide around 100,000 blood samples in the course of one and a half years. It is clear that a computerized system was needed to support data management, reporting and process control. Semi-automation is advantageous insofar as it reduces sources of error and limits manpower.
This section deals with the processing of blood samples and corresponding information, the database management system and the automated laboratory system that was designed for this study.

2 DATABASE

A relational database (based on the Relational Database Management System Informix-SQL) was designed for the TIP study. The database consists of 6 tables with records of data on:

1. personal particulars of patients
2. information on pregnancy
3. blood samples
4. laboratory tests and results
5. midwives, general practitioners and obstetricians
6. relation between patients and midwives and/or physicians.

The tables are in an 1:n relationship, except for patient and midwife, general practitioner or obstetrician (M/GP/O) tables that are linked in an m:n relationship. One or more pregnancy records are linked with a single patient, one or more samples with a pregnancy, one or more test results with a sample. A record of a patient may be linked with that of another patient in one particular situation, namely the record of a child with the record



GP/M/O general practitioner, midwife or obstetrician
* linking the record of a mother with the record of her child(ren)
** linking a sample with the GP/M/O who sent in the sample
*** linking "patients" and GP/M/O (m:n)

Figure A.1 Schematic diagram of the tables in the database

of his/her mother. Midwives, general practitioners and obstetricians are registrated in a M/GP/O or 'doctors' table that is linked to the sample table (indicating who sent in the sample). The linkage between the different records is made by means of unique indexes on specific fields; one exception is the linkage between patients and M/GP/O (m:n relationship: one 'doctor' linked with several women; one woman possibly with several 'doctors': a GP, a midwife and/or an obstetrician), which is established with a separate table. The structure of the database is shown in figure A.1.

Information from the accompanying form, shown at the end of this section, is entered into the database at the workstation. The identification number is read by a barcode pen, the other data are entered by keyboard. Data in the database on individual patients can be requested directly at the workstation. For more complicated questions statements written in SQL (Standard Query Language) have to be used. Several statements written in advance are available (see 3.3).

3 OPERATIONS

3.1 PROCESSING OF BLOOD SAMPLES AND CORRESPONDING INFORMATION

Each sample together with its accompanying form arrived in the TIP laboratory by mail in a separate package. Sample tube, accompanying form and an empty tube to be filled later on with the serum were marked with identical barcode stickers for identification. The accompanying forms were further processed by the administration unit. The blood samples were centrifuged and serum harvested in the clean tube with identical barcode. The ELISA tests were performed in polystyrene trays with 96 wells (8 rows, 12 columns). The test is semi-automated with a dispenser that adds samples or reagents to all wells at one time. Therefore the sera are stored in blocks with 96 open holders for small tubes. The blocks are loaded with serum samples at a workstation consisting of a terminal with a barcode pen and a loading device. The loading device is an instrument with photoreceptors which are aligned with the holes in the block before the sampling tubes, containing 0.5 ml serum, are put in place.
Because the first two rows of the test tray are filled with controls, these rows in the serum blocks were not filled with serum samples. Therefore

80 samples were stored in each box. For the ELISA a dispenser was used to take a fixed volume from the 80 sera and transport it to the same position on the test tray. The extinctions were read by a photometer. The technician decides whether the test was performed successfully: extinction of the control samples had to be within a predetermined range. Subsequently a command is given to the computer system to send the extinction value, the test result (+ or –) and the test date of each sample to the matching test record in the database.

3.2 PRODUCTION OF REPORTS ON TEST RESULTS

Standard letters to report the result of the screening test were written.
These letters were automatically produced by the system and the date and type of letter were included in the sample record.
Every night the system checked for blood samples with a test result but without a letter date and type; this indicated that no letter had been produced up to that time. According to preset criteria (depending on the serial number of the blood sample, the test result, the duration of pregnancy) a letter was composed. The letter was addressed to the M/GP/O who sent in the blood sample; the personal particulars, date of sample and corresponding test result were included; depending on the test result re-examination was recommended; the system also calculated the target date for the next blood sample on the basis of the duration of pregnancy of that particular woman. An accompanying form containing all available data was included. In this way letters were produced for all standard situations:

- letter type 1: a seropositive first sample with the message that the woman need not be involved in the preventive study.
- letter type 2: a seronegative first sample with the advice to repeat serological control during pregnancy (individual target date given).
- letter type 3: a seronegative repeat sample with the advice to repeat serological control during pregnancy (individual target date given).
- letter type 4: a seronegative repeat sample taken during the last trimester of pregnancy with the advice to sample cord blood at delivery.
- letter type 5/6: a seronegative cord sample with the message that no infection was traced and that the end of the study was reached for the woman concerned (one to the GP/M/O who sent in the sample, one to the woman's GP).

Moreover reminder notes (letter type 7) were produced automatically if a

sample had not been received one month after the recommended date; the GP/M/O was reminded to send in a blood sample. All letter types are printed at the end of this addendum.
Letters concerning seroconversion with a proposal for treatment and further examinations of the pregnant woman and her child were not produced by the system but on an individual base. At the end of pregnancy a letter was sent to the obstetrician to remind him of the steps of the study protocol to be performed at birth: obtain a sample of placental tissue, a blood sample from the mother and a cord sample of the baby. Accompanying forms to be sent with the samples to the RIVM were enclosed, together with a letter to inform the paediatrician to be consulted. The change from obstetric to paediatric care was a weak link in the protocol, since the paediatrician to be consulted was not always known in advance and thus could not be approached directly by the study centre. As soon as the birth of a child was reported, an appointment for ophthalmological examination was arranged by the RIVM. Results of laboratory tests and ophthalmological findings together with an interpretation and proposals for continuation of follow-up were sent by the study coordinator to the paediatrician with a copy to the general practitioner, obstetrician and/or midwife.

3.3 PROCESS MANAGEMENT

Apart from the reports on individual women the system produced information on the progress of the study: an account of the number of women, the number of blood samples received, the number of cooperating health care workers, samples without a test result, etc.
Every day the system checked for seropositive test results for women who were seronegative at earlier examination. Further action by the staff of the screening laboratory was then required: the sample together with the preceding one was offered to the diagnostic laboratory for confirmation of the suspected seroconversion.

Letter type 1

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TIP (Toxoplasma Infectie Preventie) onderzoek
tel.nr. coördinatiecentrum: 030-743018/742588

Bilthoven, <datum>

<artsnaam>
<adres>
<woonplaats>

Zeer geachte Collega,

Bij het screeningsonderzoek in het eerste bloedmonster dat werd afgenomen op <datum> van Uw patiënte (TIP-nummer:<nummer>)

<patiëntnaam>
<adres>
<woonplaats>

werden specifieke antistoffen van de IgG-klasse tegen *Toxoplasma gondii* aangetroffen. Dit is een teken van voorafgaand contact met de parasiet. Patiënte zal derhalve niet verder vervolgd worden in het kader van het TIP – Toxoplasma Infectie Preventie – onderzoek, dat zich richt op zwangeren bij wie geen aanwijzing wordt gevonden van voorafgaande infectie.

Voor nadere informatie verwijzen wij U naar het U reeds toegestuurde draaiboek en attenderen U op het telefoonnummer dat voor dit project beschikbaar is op werkdagen van 08.45 uur tot 16.45 uur: 030-743018/742588.

Met dank voor Uw medewerking,

M.A.E. Conyn-van Spaendonck, arts
projectleider.

Rijksinstituut voor Volksgezondheid en Milieuhygiëne
Postbus 1 3720 BA BILTHOVEN

TIP (Toxoplasma Infectie Preventie) onderzoek
tel.nr. coördinatiecentrum: 030-743018/742588

Bilthoven, <datum>

<artsnaam>
<adres>
<woonplaats>

Zeer geachte Collega,

Bij het screeningsonderzoek in het eerste bloedmonster dat werd afgenomen op <datum> van Uw patiënte (TIP-nummer: <nummer>)
<patiëntnaam>
<adres>
<woonplaats>
werden geen specifieke antistoffen van de IgG-klasse tegen *Toxoplasma gondii* aangetroffen. Dit betekent dat voorafgaand contact met de parasiet zeer onwaarschijnlijk is. Het is van belang een infectie van de moeder in de zwangerschap te voorkomen, in verband met het daarmee samenhangende risico op een congenitale toxoplasma infectie van haar kind.
De volgende maatregelen zijn aan te bevelen:

1. Voorlichting aan de zwangere over preventie van een toxoplasma infectie.
   Het betreft gare bereiding van vlees, goed wassen van groenten, goede keukenhygiëne, goede kattebakhygiëne (zie patiëntenvoorlichtingsfolder).
2. Herhaalde bloedafname en wel bij een zwangerschapsduur van (18), 24 en 32 weken, zodat een mogelijk alsnog optredende infectie vroeg wordt opgespoord en tot behandeling kan worden overgegaan. Hierdoor kan het risico op een infectie van het ongeboren kind worden beperkt.

Gaarne zullen wij patiënte vervolgen in het kader van het in de hele provincie Zuid-Holland georganiseerde TIP - Toxoplasma Infectie Preventie - onderzoek. Wij verzoeken U de bloedafname bij een zwangerschapsduur van <n>weken te herhalen, dus omstreeks <datum>. Bijgaand zenden wij U het aanvraagformulier voor het onderzoek toe; wij vragen U dat volledig ingevuld met het volgende bloedmonster in te zenden. Per separate post zal Uw materiaal voor bloedafname en verzending worden aangevuld. Wilt U, wanneer U de zwangerschapsbegeleiding niet zelf continueert, dit schrijven en het vooringevulde aanvraagformulier overdragen aan degene die het overneemt? Voor nadere informatie verwijzen wij U naar het U reeds toegestuurde draaiboek en attenderen wij U op het telefoonnummer dat voor dit project beschikbaar is op werkdagen van 08.45 uur tot 16.45 uur: 030-743018/ 742588.

Met dank voor Uw medewerking,

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projectleider.

Letter type 3

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Bilthoven, <datum>

<artsnaam>
<adres>
<woonplaats>

Zeer geachte Collega,

Bij het screeningsonderzoek in het bloedmonster dat werd afgenomen op <datum> van Uw patiënte (TIP-nummer: <nummer>)
<patiëntnaam>
<adres>
<woonplaats>
werden wederom geen IgG-antistoffen tegen *Toxoplasma gondii* aangetoond, zodat er ook nu geen aanwijzing is voor een infectie. Het is zaak dat patiënte de aanbevolen preventieve maatregelen blijft opvolgen. Wij verzoeken U wederom bloed af te nemen bij een zwangerschapsduur van <n>weken, dus omstreeks <datum>. Bijgaand zenden wij U het aanvraagformulier voor het onderzoek toe; wij vragen U dat volledig ingevuld in te zenden met het volgende monster. Per separate post zal Uw materiaal voor bloedafname en verzending worden aangevuld.
Voor nadere informatie verwijzen wij U naar het U reeds toegestuurde draaiboek en attenderen wij op het telefoonnummer dat voor dit project beschikbaar is op werkdagen van 08.45 uur tot 16.45 uur: 030-743018/742588.

Met dank voor Uw medewerking,

M.A.E. Conyn-van Spaendonck, arts
projectleider.

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tel.nr. coördinatiecentrum: 030-743018/742588

Bilthoven, <datum>

<artsnaam>
<adres>
<woonplaats>

Zeer geachte Collega,

Bij het screeningsonderzoek in het bloedmonster dat werd afgenomen op <datum> van Uw patiënte (TIP-nummer: <nummer>)
<patiëntnaam>
<adres>
<woonplaats>
werden wederom geen IgG-antistoffen tegen *Toxoplasma gondii* aangetoond, zodat er ook nu geen aanwijzing is voor een infectie. Het is zaak dat patiënte de aanbevolen preventieve maatregelen blijft opvolgen. Wij verzoeken U tot slot na de bevalling een monster navelstrengbloed in te zenden. Bijgaand zenden wij U het aanvraagformulier toe; wij vragen U dat volledig ingevuld in te zenden met het bloedmonster. Per separate post zal Uw materiaal voor bloedafname en verzending worden aangevuld.
Voor nadere informatie verwijzen wij U naar het U reeds toegestuurde draaiboek en attenderen wij U op het telefoonnummer dat voor dit project beschikbaar is op werkdagen van 08.45 uur tot 16.45 uur: 030-743018/742588.

Met dank voor Uw medewerking,

M.A.E. Conyn-van Spaendonck, arts
projectleider.

P.S. Aangezien het bijgesloten aanvraagformulier met het navelstrengbloed-monster van de baby zal worden ingezonden, moeten in de eerste alinea de personalia van de baby en in de derde alinea de personalia van de moeder worden ingevuld; de tweede alinea kunt u overslaan.

Letter type 5

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Bilthoven, <datum>

<artsnaam>
<adres>
<woonplaats>

Zeer geachte Collega,

Gedurende haar zwangerschap was
Uw patiënte (TIP-nummer: <nummer>)
<patiëntnaam>
<adres>
<woonplaats>
in het TIP – Toxoplasma Infectie Preventie – onderzoek betrokken. In het navelstreng-bloedmonster van haar baby (geb. datum: <ddmmjj>) werden evenals in het laatste bloedmonster afgenomen gedurende de zwangerschap bij de moeder geen specifieke antistoffen tegen *Toxoplasma gondii* aangetroffen.
Derhalve concluderen wij dat de moeder ook in de laatste zwangerschapsweken geen infectie heeft opgelopen. Hiermede is voor haar het onderzoek afgesloten. Wij verzoeken U haar dit bij de postnatale controle mee te delen.

Wij danken U hartelijk voor Uw medewerking,

M.A.E. Conyn-van Spaendonck, arts
projectleider.

Rijksinstituut voor Volksgezondheid en Milieuhygiëne
Postbus 1 3720 BA BILTHOVEN

TIP (Toxoplasma Infectie Preventie) onderzoek
tel.nr. coördinatiecentrum: 030-743018/742588

Bilthoven, <datum>

<artsnaam>
<adres>
<woonplaats>

Zeer geachte Collega,

Gedurende haar zwangerschap was
Uw patiënte (TIP-nummer: <nummer>)
<patiëntnaam>
<adres>
<woonplaats>
in het TIP – Toxoplasma Infectie Preventie – onderzoek betrokken. In het navelstreng-bloedmonster van haar baby (geb. datum: <ddmmjj> werden evenals in het laatste bloedmonster afgenomen gedurende de zwangerschap bij de moeder geen specifieke antistoffen tegen *Toxoplasma gondii* aangetroffen.
Derhalve concluderen wij dat de moeder ook in de laatste zwangerschapsweken geen infectie heeft opgelopen. Hiermede is voor haar het onderzoek afgesloten. Wij verzoeken U haar dit bij de postnatale controle mee te delen.

Wij danken U hartelijk voor Uw medewerking,

M.A.E Conyn-van Spaendonck, arts
projectleider.

Letter type 7

Rijksinstituut voor Volksgezondheid en Milieuhygiëne
Postbus 1 3720 BA BILTHOVEN

TIP (Toxoplasma Infectie Preventie) onderzoek
tel.nr. coördinatiecentrum: 030-743018/742588

Bilthoven, <datum>

<artsnaam>
<adres>
<woonplaats>

Zeer geachte Collega,

Gezien het feit dat Uw patiënte (TIP-nummer: <nummer>)
<patiëntnaam>
<adres>
<woonplaats>
blijkens bloedonderzoek bij zwangerschapsduur van <n> weken niet eerder een toxoplasma infectie heeft doorgemaakt, adviseerden wij U bij een zwangerschapsduur van <n> weken, dus omstreeks <datum>, opnieuw een bloedmonster te laten onderzoeken. Ons laboratorium heeft dit monster echter tot op heden nog niet ontvangen. Wij raden U aan alsnog bloed af te nemen en aan ons toe te zenden. Bijgaand zenden wij U opnieuw een aanvraagformulier voor het onderzoek dat volledig ingevuld met het bloedmonster kan worden meegestuurd. Wordt van verdere deelname aan het TIP-onderzoek afgezien, dan zouden wij graag over de reden daarvan geïnformeerd worden.

Voorts wijzen wij U op de mogelijkheid tot nader overleg waartoe het volgende telefoonnummer beschikbaar is op werkdagen van 08.45 uur tot 16.45 uur: 030-743018/742588.

Met dank voor Uw medewerking,

M.A.E. Conyn-van Spaendonck, arts
projectleider.

Rijksinstituut voor Volksgezondheid en Milieuhygiëne
Postbus 1 3720 BA BILTHOVEN

TIP (Toxoplasma Infectie Preventie) onderzoek
tel.nr. coördinatiecentrum: 030-743018/742588

Bloedmonster afgenomen door huisarts/verloskundige/gynaecoloog*

d.d. ☐☐ - ☐☐ - ☐☐ (dag-maand-jaar) van:
Naam**:
Eerste voornaam:
Voorletters van de overige namen:
Geslacht: Geboortedatum: (dag-maand-jaar)
Gehuwd met:
Adres:
Postcode:
Woonplaats:

Alleen invullen als het bloedmonster van een zwangere is: Datum waarop
de laatste menstruatie begon: (dag-maand-jaar) Gravida ☐☐ Para ☐☐ Abortus ☐☐

Alleen invullen als het bloedmonster van een kind is:
Meisjesnaam v.d. moeder:
Voorletters:
Gehuwd met:
Geboortedatum van de moeder: (dag-maand-jaar)
Gaarne opgave van de huisarts en de betrokken verloskundige en/of gynaecoloog:

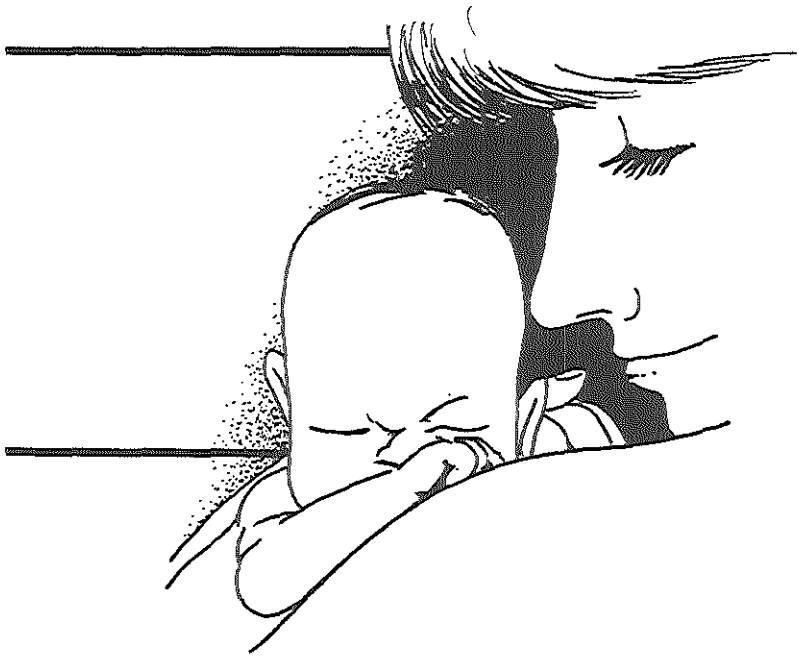
Huisarts
Naam:
Voorletters:
Praktijkadres:
Postcode:
tel.nr:
Verloskundige
Naam:
Voorletters:
Praktijkadres:
Postcode:
tel.nr:
Gynaecoloog
Naam:
Voorletters:
Praktijkadres:
Postcode:
tel.nr:
Betreft het een tweelingzwangerschap: ja/nee.
Andere bzonderheden:

* Doorstrepen hetgeen niet van toepassing is.
** Bij gehuwde vrouwen meisjesnaam.
Betreft het onderzoek van een baby, dan hier de personalia van het kind invullen.

Appendix 1a

Information leaflet for participants to the TIP study concerning primary and secondary prevention of toxoplasma infection.

In verwachting?



Voorkom dan een toxoplasma infectie

Wat is een toxoplasma infectie

Toxoplasma gondii is de naam van een microscopisch klein diertje, dat men parasiet noemt. Deze parasiet kan mensen infecteren. Meestal zijn er bij zo'n infectie geen ziekteverschijnselen, maar als dat wél het geval is, zijn de klachten vrij vaag, bijvoorbeeld moeheid, lusteloosheid, klierzwellingen, lichte koorts, huiduitslag. Men spreekt dan van **toxoplasmose**. Over het algemeen is de ziekte binnen enkele weken of maanden helemaal over. En: wie eenmaal, al dan niet bewust, een toxoplasma infectie heeft gehad, is daarna immuun (onvatbaar) voor de parasiet. Tot zover zijn de problemen wel vervelend maar niet onoverkomelijk.

Het wordt echter heel anders — en gevaarlijker — als een vrouw tijdens haar zwangerschap voor de eerste keer met toxoplasma besmet wordt. Het gevaar geldt niet zozeer de aanstaande moeder, maar juist het ongeboren kind. Het kind kan via de moeder besmet raken en dáárdoor ernstige afwijkingen krijgen, met name van het zenuwstelsel (waterhoofd) en de ogen (blindheid). Men spreekt dan van **congenitale (aangeboren) toxoplasmose**. De ernst van de afwijkingen is afhankelijk van het tijdstip in de zwangerschap waarop de moeder wordt geïnfecteerd en dus van het ontwikkelingsstadium waarin de baby zich bevindt. Soms treedt abortus of vroeggeboorte op. De verschijnselen van zo'n aangeboren toxoplasma infectie behoeven niet direct zichtbaar te zijn. Soms komen ze pas op latere leeftijd aan het licht; dit betreft dan vaak oogklachten.

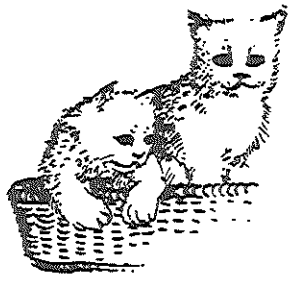
Gelukkig wordt de toxoplasma parasiet tijdens de zwangerschap niet altijd van de moeder op het kind overgedragen. Men vermoedt dat dit gebeurt in vier van de tien gevallen.

Hoe ontstaat besmetting met toxoplasma

Landbouwhuisdieren zoals varkens, schapen en koeien kunnen met toxoplasma besmet zijn zonder dat zij ziekteverschijnselen tonen. De toxoplasma parasiet kan dan in het vlees aanwezig zijn. Door het eten van dat vlees, als dat rauw of onvoldoende verhit is, kan de ziekteverwekker levend de mens bereiken.

Verder is bekend dat in de ontlasting van vooral jonge katten tijdelijk toxoplasma parasieten kunnen zitten. Deze parasieten zijn pas na enkele dagen gerijpt en in staat om te infecteren. Daarom is het belangrijk dat de kattebak dagelijks verschoond wordt, zodat de parasieten verwijderd worden voordat ze gerijpt zijn. Ook onze omgeving (tuin, straat) kan via katteuitwerpselen besmet zijn. Het is dus ook belangrijk om bij tuinwerk handschoenen te gebruiken en groenten die mogelijk besmet zijn, vóór consumptie goed te wassen.
Een infectie door direct contact met dieren is onwaarschijnlijk.

In Nederland hebben naar schatting vier van de tien vrouwen vóór het bereiken van de geslachtsrijpe leeftijd ooit een toxoplasma infectie gehad. Voor de andere vrouwen (zes van de tien) is het dus erg belangrijk dat zij voorkómen tijdens hun zwangerschap besmet te raken met toxoplasma. Immers tijdens de gehele zwangerschap blijft er een risico bestaan dat het ongeboren kind via de moeder besmet wordt.



Preventie onderzoek Zuid-Holland

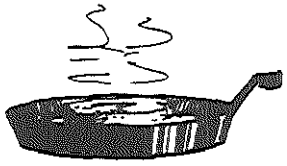
In januari 1987 wordt begonnen met het Toxoplasma Infectie Preventie (TIP)-onderzoek in de provincie Zuid-Holland. Doel is het effect van preventie te meten en de meest geschikte wijze van uitvoering van het preventieve programma vast te stellen. Het onderzoek wordt — in samenwerking met Uw arts, verloskundige of gynaecoloog — in opdracht van de Geneeskundige Hoofdinspectie gedaan door het Rijksinstituut voor Volksgezondheid en Milieuhygiëne (RIVM).

De bedoeling is om, samen met U, te verhinderen dat Uw kind aangeboren toxoplasmose krijgt. Er zijn voor U geen kosten aan het onderzoek verbonden.

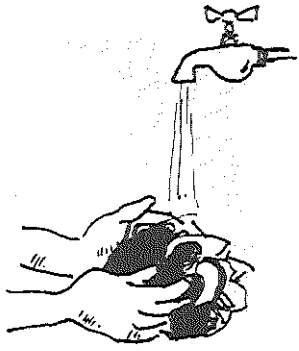
Wat wordt er van U verwacht

- ☐ Als U aan het proef-onderzoek wilt meedoen dan zal om te beginnen van U worden gevraagd om, zo vroeg mogelijk in Uw zwangerschap, een bloedmonster af te staan.
- ☐ Als uit het onderzoek blijkt dat U eerder in Uw leven een toxoplasma infectie heeft doorgemaakt, dan is verdere controle niet nodig.
- ☐ Als uit het bloedonderzoek naar voren komt dat U nog nooit contact heeft gehad met de toxoplasma parasiet, wordt het anders. In dat geval zal op de eerste plaats alles moeten worden gedaan om een infectie te voorkomen. Volgt onze adviezen daarom goed op!
- ☐ Elke acht weken zal Uw bloed worden gecontroleerd. Degene die Uw zwangerschap begeleidt, zal telkens één buisje bloed afnemen door een prik in de elleboogholte. Tot slot wordt een beetje bloed van Uw kind uit de navelstreng in een buisje opgevangen voor onderzoek.

Advies

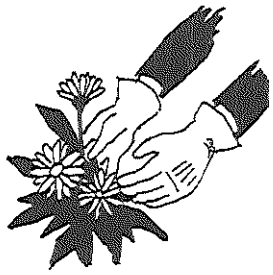


1



2

1. Eet tijdens Uw zwangerschap geen rauw of halfrauw vlees. Verhit al het vlees door en door! Goed gebraden of gestoofd vlees vormt geen enkel risico; "rood" vlees wel!



3

2. Was alle groenten terdege!

3. Draag handschoenen als U in de tuin werkt!

4. Kom niét aan de kattebak; laat de kattebak dagelijks door iemand anders schoonmaken!

4

U zult begrijpen dat deze vier adviezen ook belangrijk zijn voor iedere zwangere vrouw die niet aan het onderzoek deelneemt.

En hoe nu verder...

Voor de meesten van U wordt het onderzoek afgesloten na de geboorte van een gezond kindje.
De vier genoemde maatregelen kunnen echter, hóe verstandig dan ook, niet voor 100% garanderen dat U geen toxoplasma infectie krijgt. Indien uit het herhaalde bloedonderzoek blijkt dat U de infectie toch heeft opgelopen, dan is het raadzaam om snel te beginnen met een behandeling met medicijnen. Die behandeling kan het gevaar beperken dat de infectie op Uw kind overgaat. Kort na de geboorte en enkele malen in het eerste levensjaar zal Uw kind uitgebreid door kinderarts en oogarts worden onderzocht om uit te maken of het geïnfecteerd is en of het zélf nog enige tijd met medicijnen moet worden behandeld.
Dankzij Uw medewerking kan inzicht verkregen worden of dit preventieve programma voldoet en of het zal kunnen worden aangeboden aan alle vrouwen in Nederland die zwanger zijn of willen worden.

Heeft U nog vragen

Bespreek eventuele vragen altijd met Uw arts of verloskundige. Bij problemen kan door deze altijd overleg gevoerd worden met het RIVM.

TIP Toxoplasma Infectie Preventie-onderzoek

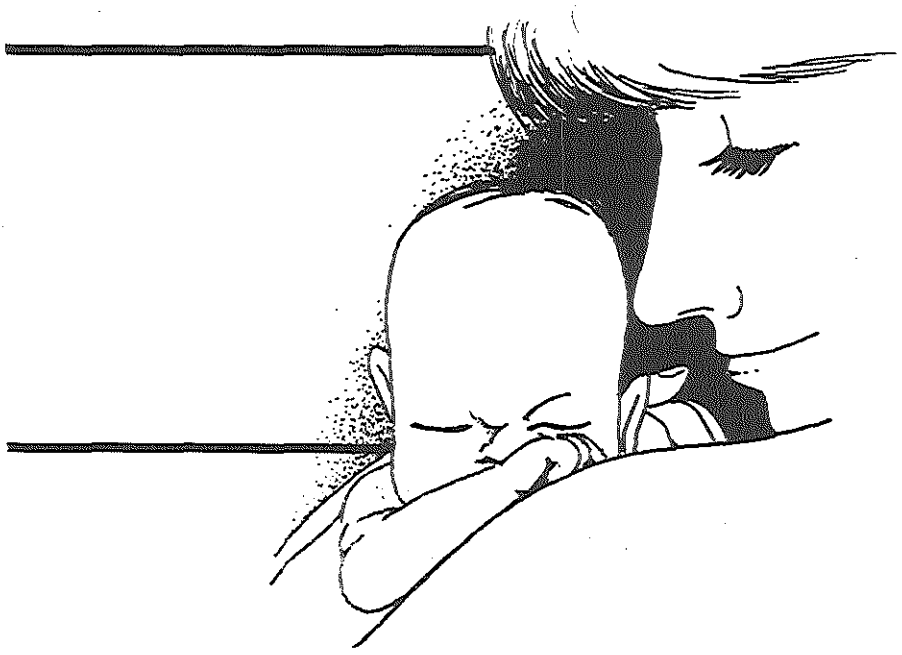
rivm **Rijksinstituut voor de Volksgezondheid en Milieuhygiëne**
Antonie van Leeuwenhoeklaan 9
Postbus 1 • 3720 BA Bilthoven • Tel. 030-749111

GHI **Geneeskundige Hoofdinspectie van de Volksgezondheid**
Postbus 5406 • 2280 HK Rijswijk • Tel. 070-407911

Appendix 1b

Second version of information leaflet for women of child-bearing age concerning primary prevention of toxoplasma infection.

In verwachting?



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Het wordt echter heel anders — en gevaarlijker — als een vrouw tijdens haar zwangerschap voor de eerste keer met toxoplasma besmet wordt. Het gevaar geldt niet zozeer de aanstaande moeder, maar juist het ongeboren kind. Het kind kan via de moeder besmet raken en dáárdoor ernstige afwijkingen krijgen, met name van het zenuwstelsel (waterhoofd) en de ogen (blindheid). Men spreekt dan van **congenitale (aangeboren) toxoplasmose.** De ernst van de afwijkingen is afhankelijk van het tijdstip in de zwangerschap waarop de moeder wordt geïnfecteerd en dus van het ontwikkelingsstadium waarin de baby zich bevindt. Soms treedt een miskraam of vroeggeboorte op. De verschijnselen van zo'n aangeboren toxoplasma infectie behoeven niet direct zichtbaar te zijn. Soms komen ze pas op latere leeftijd aan het licht; dit betreft dan vaak oogklachten.

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Hoe ontstaat besmetting met toxoplasma

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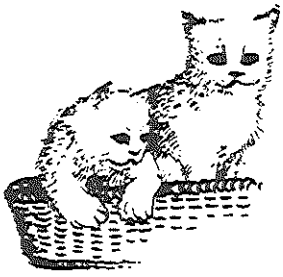
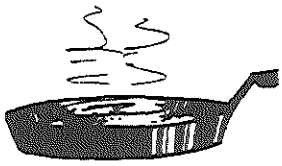
Verder is bekend dat in de ontlasting van vooral jonge katten tijdelijk toxoplasma parasieten kunnen zitten. Deze parasieten zijn pas na enkele dagen gerijpt en in staat om te infecteren. Daarom is het belangrijk dat de kattebak dagelijks verschoond wordt, zodat de parasieten verwijderd worden voordat ze gerijpt zijn. Ook onze omgeving (tuin, straat) kan via katteuitwerpselen besmet zijn. Het is dus ook belangrijk om bij tuinwerk handschoenen te gebruiken en groenten die mogelijk besmet zijn, vóór consumptie goed te wassen.
Een infectie door direct contact met dieren is onwaarschijnlijk.

In Nederland zullen veel vrouwen nog geen toxoplasma infectie hebben doorgemaakt. Daarom is het erg belangrijk voor zwangeren dat zij voorkomen tijdens hun zwangerschap besmet te raken met toxoplasma. Immers tijdens de gehele zwangerschap blijft er een risico bestaan dat het ongeboren kind via de moeder besmet wordt.
Een proefonderzoek dat in 1987 in Zuid-Holland werd uitgevoerd, leidde tot de voorlopige conclusie dat bloedonderzoek, om uit te maken wie al een infectie heeft gehad of om op te sporen wie een infectie doormaakt, niet aangeraden hoeft te worden.
Er wordt al een groot voordeel verwacht van voorlichting aan zwangeren hoe zij zelf kunnen proberen een infectie te voorkomen.



Advies



1

2

1. Eet tijdens Uw zwangerschap geen rauw of halfrauw vlees. Verhit al het vlees door en door! Goed gebraden of gestoofd vlees vormt geen enkel risico; "rood" vlees wel!

3

2. Was alle groenten terdege!

3. Draag handschoenen als U in de tuin werkt!

4. Kom niét aan de kattebak; laat de kattebak dagelijks door iemand anders schoonmaken!

4

TIP

Toxoplasma Infectie Preventie-onderzoek

rivm

Rijksinstituut voor Volksgezondheid en Milieuhygiëne
Antonie van Leeuwenhoeklaan 9
Postbus 1 • 3720 BA Bilthoven • Tel. 030-749111

GHI

Geneeskundige Hoofdinspectie van de Volksgezondheid
Postbus 5406 • 2280 HK Rijswijk • Tel. 070-407911

Appendix 1b

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DANKWOORD

Het laat zich raden dat een onderzoek onder 28.000 zwangeren en hun kinderen op de inzet van veel mensen draait; de verloskundigen, huisartsen en vrouwenartsen in de provincie Zuid Holland hebben naast hun drukke praktijkwerkzaamheden belangeloos hun medewerking aan het TIP onderzoek gegeven; zonder hun inzet zou nooit zoveel informatie vergaard zijn.

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