Toxoplasma infection among pregnant women in Norway; susceptibility, diagnosis and follow-up

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Series of dissertations submitted to the
Faculty of Medicine, University of Oslo

ISBN 978-82-8377-037-7

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Cover: Hanne Baadsgaard Utigard.
Print production: Reprosentralen, University of Oslo.

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Preface

Travelling in Europe with my family as a child and watching church steps as part of my mother's degree in art history, my academic seed was planted. Though, coloured by interesting dinnertime stories from my father's dentist practice, the field of medicine became my dream and goal from 13 years of age.

My journey into research started with parental influence, but has been closely linked to my clinical work in gynaecology and obstetrics. After being a passionate globetrotter, receiving a diploma in tropical medicine and working in Bangladesh, I got in contact with Babill Stray-Pedersen whose field of work was a materialization of my strongest interests; maternal and foetal health in combination with the interesting field of infectious medicine. She introduced me to the world of *Toxoplasma gondii*. As she did her thesis on the parasite during the seventies, she decided to complete the cycle of her academic carrier by tutoring a new thesis on the topic.

Congenital toxoplasmosis has received little focus in Norway in the last 10 years, after the research of Pål Jenum during the nineties and we have no updated estimate on the prevalence of toxoplasma IgG among pregnant women. Toxoplasma infection may be considered as one of the "neglected" congenital diseases.

We therefore decided to bring the old knowledge into consciousness once more, perform a new prevalence study and analyse our patient population of women with amniocentesis on indication suspected toxoplasma infection.

Acknowledgements

The present work was carried out at the Division of Gynaecology and Obstetrics at Rikshospitalet, Oslo University Hospital in collaboration with the Department of Medical Microbiology at Oslo University Hospital and Department of Laboratory Medicine, Section of Medical Microbiology, Vestre Viken Hospital Trust. The first part of the project was performed in collaboration with the Norwegian Institute of Public Health.

As a research fellow, the funding and salary has been acquired through The University of Oslo, Institute of Clinical Medicine as my employer. Vestre Viken Hospital Trust funded the analyses of the toxoplasma serology in the first part of the project.

Firstly, I am grateful to Professor Babill Stray-Pedersen for suggesting the post as a research fellow at the Institute of Clinical Medicine and for introducing the world of *Toxoplasma gondii* and the joy of teaching medical students. Above all, special thanks for being my main supervisor and the project leader. Despite age, she has a burning desire for research in the developing world in the field of mother and child health.

Secondly, warm thanks to my supervisor Pål Jenum, for sharing his expertise and knowledge and for replying to all my mails, correcting, correcting and correcting again my papers despite an overfilled calendar.

Thirdly, I`m very grateful to Guttorm Haugen for being a warm and supportive leader, a knowledgeable co-author and collaborative partner with good ideas, advises and constructive critics.

Special thanks to;

Thomas Åbyholm, Kristi Hjelle and Tone Berge for allowing me access to the patient material in the second part of the project.

Susanne Dudman for including me in their immunity project, allowing us to use sera and data collected by them.

My co-authors for valuable comments on my papers and for their valuable contributions in their fields of expertise, in particular, Anne Helbig with her German and academic thoroughness and valuable comments from the daily practice as an expert in foetal medicine dealing with antenatal infections, and Irene Sandven for helping out with the statistical part of the cross-sectional study.

My collaborating partners; Tone Berge at The Toxoplasma Reference Laboratory for thoroughly informing me and updating me on the different laboratory methods. The laboratory staff, Ellen Eikum

and Mette Andersen Holthe for analysing and reanalysing toxoplasma antibodies at the Department of Laboratory Medicine, Section of Medical Microbiology, Vestre Viken Hospital Trust.

My fellow PhD-students and colleagues, Ingvil, Anne, Hans Kristian, Trond, Marie Cecilie, Lina, Pawel, Gun Lisbeth, Ane, Hildegunn, Maya, Katharina and Synne for stimulating discussions, inputs, shared frustrations and joys during Friday lunches and ice cream in the sun.

Our very inspiring teacher in statistics Kathrine Frey Frøslie for breaking down medical statistics in to understandable pieces and for enthusiastically answering the same questions year after year.

My colleagues at Rikshospitalet for letting me take part in their interesting daily activities, as a teacher and doctor. Thank you all for making my job of teaching students at the labour ward easy, interesting and a pleasure.

Pernille Frese for always being helpful and in particular helping out with editing posters and the thesis.

Finally, to my family: Thanks to my dear Torfinn for being a steady trustable co-captain helping me navigate through the choppy seas of our small family, for believing in me, giving support and helping out with graphics and design.

Thank to my mother who introduced me to academia, for being supportive and for giving scientific advices. Above all, thanks for being a good mother and grandmother “besta”.

Thanks to my father and his Tone and mother (mor) and father (far) for being whom you are, caring for our children and for giving them valuable memories. Thanks to my sisters Siri and Kristin and their lovely families for participating in our lives and for being an important part of our family team.

Warm hugs to my dearest children Elvira and Even, for bringing me back to reality, distracting me from all the scientific problems, giving me the best hugs ever (“med begge henna”) and for handing me the rainbow…

Thanks to all my friends “outside” for reminding me that life exist elsewhere, especially Cili for uncomplicated coexistence at Bertebu, creating many wonderful memories.

Abbreviations

AC	Amniocentesis
AI	Avidity index
CI	Confidence interval
CMIA/CLIA	Chemiluminescence microparticle enzyme-immunoassay
CV%	Coefficient of variation
EIA	Indirect enzyme-immunoassay
ELISA	Enzyme-Linked Immunosorbent Assay
GA	Gestational age
GW	Gestational weeks
IgA	Immunoglobulin A
IgM	Immunoglobulin M
IgG	Immunoglobulin G
ISAGA	ImmunoSorbent Agglutination Assay
MEIA	Microparticle enzyme-immunoassay
OR	Odds ratio
PCR	Polymerase chain reaction
T. gondii	*Toxoplasma gondii*
Toxo IgG	Toxoplasma immunoglobulin G antibody

Definitions

Avidity= Avidity measures the overall binding strength between the multivalent antigen and antibodies. (http://www.microbiologybook.org/mobile/m.immuno-7.htm)

CV%= Coefficient of variation. The coefficient of variation is a measure of distribution that describes the variability around the mean (http://support.minitab.com/en-us/minitab/17/topic-library/basic-statistics-and-graphs/summary-statistics/what-is-the-coefficient-of-variation).

Epidemiological

Incidence = The number of individuals falling ill (new cases), during a given period in a specified population (1).

Period prevalence = *"The total number of individuals who have an attribute or disease at a particular time divided by the population at risk of having the attribute or disease at a specified period of time"* (1).

Prevalence = *"The total number of individuals who have an attribute or disease at a particular time divided by the population at risk of having the attribute or disease at that time"* (1).

Positive predictive value = The probability that a person with positive test result is true positive (e.g. does have the disease) (1).

Negative predictive value = The probability that a person with negative test result is true negative (e.g. does not have the disease) (1).

Sensitivity = *"Sensitivity is the probability that a diseased person (case) in the population tested will be identified as diseased by the test "(1).*

Specificity = *"Specificity is the probability that a person without the disease (non-case) will be correctly identified as non-diseased by the test" (1).*

Microbiological

Apicomplexan: A large group (phylum) of protozoans that are characterized by having a special organelle called an apical complex helping to invade the animal cell. Most of them are single-celled, parasitic, and spore-forming. (http://www.biology-online.org/dictionary/Apicomplexa).

Bradyzoite: Slow-growing form of a microorganism. In latent toxoplasma infection, bradyzoites microscopically present as clusters enclosed by a wall (pseudocyst) in infected tissue (https://www.wikipedia.org/wiki/Apicomplexan_life_cycle).

Coccidian: A member of a group of one-celled parasites that infect the epithelial (lining) cells of the intestinal tract. (http://www.cdc.gov/parasites/glossary.html)

Protozoa: Eukaryotic (membrane coated nucleus) single-celled organisms, most of them motile (http://www.biology-online.org/dictionary/Protozoa)

Oocyst: A thick walled structure containing the zygote (a fertilized egg) of an apicomplexan parasite (2). The oocyst contains and releases the infective sporozoites.

Sonicate: To expose to sound waves; to disrupt or cut bacteria into pieces by exposure to high-frequency sound waves (http://www.medical-dictionary.thefreedictionary.com/**sonicate)**.

Sporozoite: The motile spore-like stage of some parasites, which is infective (https://en.oxforddictionaries.com/definition/sporozoite).

Sporulation: Refers to the process of forming spores. Spores allow bacteria to survive in extreme conditions, including very dry, acidic or cold conditions (https://www.reference.com/science/process-sporulation).

Tachyzoite: A motile and rapidly multiplying (proliferative) stage of a microorganism (http://www.medilexicon.com/medicaldictionary).

1 List of papers

I. Findal G, Barlinn R, Sandven I, Stray-Pedersen B, Nordbø SA, Samdal HH, Vainio K, Dudman SG, Jenum PA.
Toxoplasma prevalence among pregnant women in Norway: a cross-sectional study.
APMIS. 2015;123(4):321-5.

II. Findal G, Stray-Pedersen B, Holter EK, Berge T, Jenum PA.
Persistent low toxoplasma IgG avidity is common in pregnancy: experience from antenatal testing in Norway.
PLoS One. 2015;10(12):e0145519.

III. Findal G, Helbig A, Haugen G, Jenum PA, Stray-Pedersen B.
Management of suspected primary *Toxoplasma gondii* infection in pregnant women in Norway: twenty years of experience of amniocentesis in a low-prevalence population.
Submitted to BMC Pregnancy and Childbirth, 24 August 2016.

Table 1. Overview of papers included in this thesis

	Paper I	Paper II	Paper III
Aims	To estimate the toxoplasma IgG prevalence among pregnant women in Norway and to compare the results with the prevalence found in Norwegian studies conducted in 1974 and 1992. .	To examine the development of the toxoplasma IgG avidity in pregnant women with the focus on duration of low avidity.	To evaluate the use of amniocentesis to diagnose foetal toxoplasma infection with the aim of determining whether amniocentesis is performed on the correct patients and whether the procedure is safe for this indication.
Group	1. Pregnant women in Buskerud county 2. Pregnant women in Sør-Trøndelag county	Pregnant women undergoing amniocentesis due to suspected toxoplasma infection, with repeated avidity measurements in pregnancy	Women with singleton pregnancies going through amniocentesis because of suspected toxoplasma infection
Sample size	1. n=992 2. n=930	n= 176	n= 346
Design	Cross-sectional	Retrospective cohort	Retrospective cohort
Main explanatory variables	• County • Maternal age	• Maternal age • GA at serology • Serology profile • Foetal infection status • IgG avidity value	• Serologic group • GA at toxoplasma serology • GA at AC • Volume of amniotic fluid removed • PCR-answer • Clinical findings
Outcome	Toxoplasma IgG antibody status IgG antibody value (IU/ml)	IgG avidity development	Time of maternal infection Miscarriages Foetal infection
Main results	The general prevalence was 9.3%. The prevalence of toxoplasma IgG in Buskerud was 8.3%. The prevalence in Sør-Trøndelag was 10.4%.	139 (79%) women with IgG avidity below high threshold ≥ 3 months 74 (42%) women had stable low avidity≥ 3 months	50% (173) of the women was considered to be infected prior to pregnancy and 27% (95) during pregnancy. 15 offspring was diagnosed with congenital toxoplasmosis, 14 with positive PCR in amniotic fluid. Two miscarriages occurred four weeks after amniocentesis, both performed in GW 13.

2 Summary

Maternal infection with the parasite *Toxoplasma gondii* may cause severe damage to the offspring if the infection is transmitted through the placenta. Maternal infection is examined serologically by toxoplasma antibodies. Primary infection is suspected if the *Toxoplasma* IgG antibody level is high, IgM is positive and the IgG avidity is low. Latent infection is suspected by IgG positivity, high IgG avidity and IgM negativity (most often). To diagnose possible foetal infection in pregnancy, amniocentesis with toxoplasma PCR is performed and follow-up and toxoplasma testing at birth is initiated.

The prevalence of toxoplasma IgG antibodies among pregnant women, hence the maternal immunity, varies widely in the world. The last prevalence study on toxoplasma IgG antibodies among pregnant women in Norway took place in 1992-93. Despite increase in risk behaviour we do not have an updated prevalence to serve as background for our antenatal advises. The last 24 years we have performed amniocentesis on indication suspected toxoplasma infection, but we have never analysed our material in order to assess whether we do amniocentesis on the right patients and whether the procedure is safe on this indication. Most studies on follow-up in pregnancy are carried out in high toxoplasma IgG prevalence countries, or in countries where toxoplasma screening is performed.

The main topic of the present thesis is antenatal infection caused by the parasite *Toxoplasma gondii* with emphasis on epidemiology, handling and diagnostics of possible maternal toxoplasma infection.

The project contains two main parts; first part is a cross-sectional period prevalence study on the prevalence of *Toxoplasma gondii* IgG positivity among pregnant women in the Norwegian counties Buskerud and Sør-Trøndelag (paper I). The second part of the project is a retrospective cohort study describing the population of pregnant women undergoing amniocentesis due to suspected toxoplasma infection at Oslo University Hospital between 1992 and 2013 (paper III). As a sub study in the second part of the project we looked at all women in our cohort with low IgG avidity and serial avidity tested serum samples over a period of three months or longer (paper II).

Aims

Paper I: To estimate the toxoplasma IgG prevalence among pregnant women in Norway and to compare the results with the prevalence found in Norwegian studies conducted in 1974 and 1992.

Paper II: To examine the development of the toxoplasma IgG avidity in pregnant women with the focus on duration of low avidity.

Paper III: To evaluate the use of amniocentesis to diagnose foetal toxoplasma infection with the aim of determining whether amniocentesis is performed on the correct patients and whether the procedure is safe for this indication.

Material and methods

Paper I: This study was part of a research programme initiated by the Norwegian Institute of Public Health, "Immunity in the Norwegian population". Sera from 2000 pregnant women in Buskerud (999) and Sør-Trøndelag counties (1001) in Norway were collected consecutively. The presence of toxoplasma IgG was identified by values ≥8 IU/mL using an ELISA test performed at the Department of Laboratory Medicine, Section of Medical Microbiology, Vestre Viken Hospital Trust during the autumn 2012 and the winter 2013.

Paper II: As part of the study mentioned below, serial blood samples from 176 pregnant women were included. The criteria of inclusion were low IgG avidity and serial serum samples collected and analysed over a time span of three months or longer. The toxoplasma IgG avidity method used was based on Platelia Toxo IgG assay.

Paper III: In this retrospective study we included all singleton pregnancies (346) that underwent amniocentesis as part of prenatal diagnostics at Oslo University Hospital due to suspected primary *T. gondii* infection from 1 September 1992 to 31 December 2013. Maternal and foetal information were mainly obtained from clinical hospital records, laboratory records and antenatal health cards.

Main results

Paper I: The overall prevalence of toxoplasma IgG seropositivity was 9.3% (95% CI 8.1–10.7). In Sør-Trøndelag county the prevalence was 10.4% (95% CI 8.6–12.6) and in Buskerud county 8.3% (95% CI 6.7–10.2). There was no difference between the counties ($p = 0.13$), and the result did not differ from prevalence's found in 1975 (12.1%) and 1994 (10.7%). We found a higher prevalence among women ≥40 years compared to younger women (OR 2.65, 95% CI 1.30–5.42).

Paper II: In 139 (79%) of the included women the toxoplasma IgG avidity remained below the high threshold ≥ 3 months. Within this group 74 (74/176, 42%) women had stable low IgG avidity during the observation period and 62 (62/176, 35%) had significant increase in avidity without reaching high threshold. In 37 (37/176, 21 %) women only, the avidity increased from low to high in < 3 months. Median gestational age at first toxoplasma serology was 10.6 weeks (range 4.6–28.7).

Paper III: Retrospectively we evaluated 173 women (50%) to have been infected before pregnancy, 80 (23%) possibly during pregnancy and 93 (27%) to have been infected during pregnancy. In total 15 (4.3%) infants were considered infected with the toxoplasma parasite and among them 14 were toxoplasma PCR positive in the amniotic fluid (93%). Two miscarriages occurred after amniocentesis, both performed in GW 13.

Main conclusions

The prevalence of toxoplasma IgG in pregnant Norwegian women has been stable, but low the last 40 years despite an increase in risk behaviour and risk factors known in the literature. Around 50% of the women going through amniocentesis in our project most likely were infected prior to conception. In 79% of the cases the IgG avidity persisted low for three months or longer. The first toxoplasma serology in pregnancy is sampled at the end of first trimester leaving the physicians with the difficult task of deciding whether further prenatal diagnostics and follow-up is indicated. However, if indicated, amniocentesis after 15 GW seems safe and useful as a diagnostic procedure to diagnose congenital toxoplasma infection.

3 Introduction

3.1 The history and general introduction

Toxoplasma gondii (T. gondii) (toxon=”bow” in Greek, plasma=creature, gondii=the African rodent “gundi”) is a protozoan parasite that can infect all warm-blooded animals, including humans. The parasite was first described by Laveran in 1900 and was found in the liver and spleen of North African rodents (*Ctenodactylus gundi)* by Nicolle and Manceaux in 1908 (3, 4). The same year Splendore described the organism in rabbit tissue and Darling described the first case in humans (5-7). In 1923 parasitic cysts were found on the retina in an 11-month-old child with congenital hydrocephaly and micropthalmia, described by the ophthalmologist Janku (5). Levaditi suggested a possible connection between congenital hydrocephaly and the parasite and was among the first to report that tissue cysts persist for months (5). Kean et al. were probably the first to describe congenital toxoplasma infection and in 1939 infection with *T. gondii* was established as a prenatal transmitted disease (8). In 1942, Sabin described the characteristic symptoms and signs of congenital toxoplasmosis; the classic tetrad with chorioretinitis, hydrocephaly, convulsions and intracerebral calcifications (9).

Sulphonamides were discovered to have antitoxoplasma effect in mice in 1942. In 1952 pyrimethamine was shown to protect mice from infection (10, 11). The drugs were observed to act synergistically (12). In 1958 the macrolide spiramycin was found to have an antiparasitic effect (13).

Sabin and Feldman originated a serologic test, the dye test, in 1948 to diagnose acute toxoplasma infection (14). In 1969 the life cycle of the parasite was reported and the cat was found to be the definitive host (15).

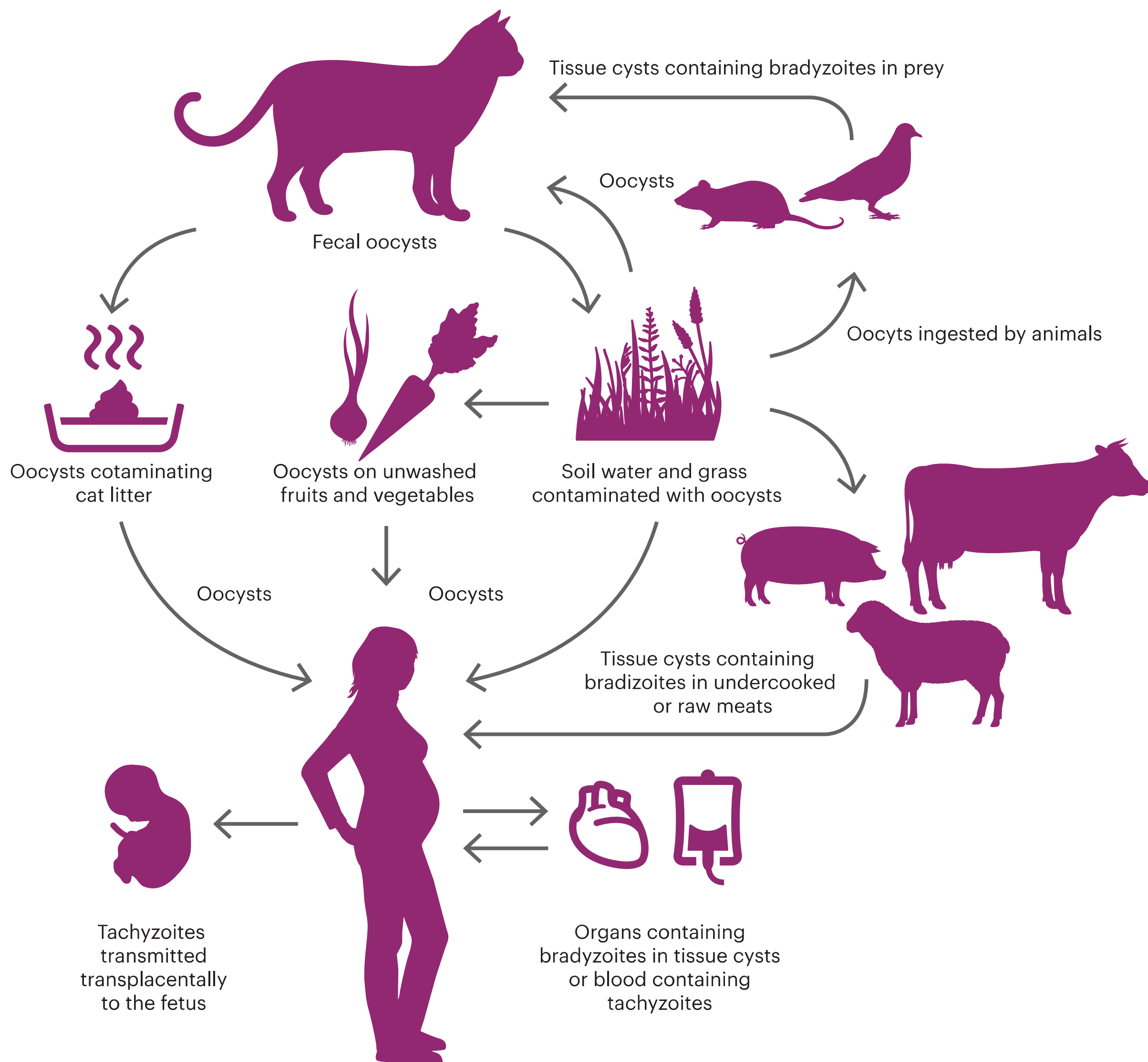
The first case of congenital toxoplasma infection in Sweden was reported in 1947, in Denmark in 1948 and in Norway by Standal and Kåss in 1952 (16-18). During the seventies, Stray-Pedersen conducted studies on toxoplasma infection in pregnancy and infancy; and during the nineties Jenum did his research on diagnosis and epidemiology of *T. gondii* among pregnant women in Norway (19-25).

3.2 The organism

T. gondii is a coccidian parasite and a member of the phylum Apicomplexa. Felines (the cat family) are the only hosts where sexual development (formation of oocysts), occurring in the intestinal epithelium, is known to take place. Millions of infectious oocysts are shed in the cat

faeces, in a period from 7 to 20 days after infection (5). Outside the cat, the parasite has three forms: an *oocyst* in which the sporozoites are formed, a proliferative form (the *tachyzoite*) and a tissue pseudocyst form containing *bradyzoites* (5).

Figure 1. The lifecycle and transmission routes of *Toxoplasma gondii* in animals and humans



The fully sporulated oocyst is infective. The sporulation (asexual development), is dependent on a temperature between 4 and 37 ˚. Temperatures above 60˚ and below minus 20˚will kill the oocyst(26). The parasites (bradyzoites or sporozoites) invade the enterocytes of the intestines of the host after being ingested (27, 28). Within the cytoplasm of the host cell, the sporozoite or bradyzoite develop into tachyzoites. The tachyzoites replicate within the host cells until the cell becomes distended with parasites and ruptures (5, 6, 27, 29). From here the

tachyzoites easily spread through lymphatic tissue and blood to all organs. The tachyzoites are only seen in the acute stage of the infection. Because of the immune response by the host, the tachyzoite soon forms semi-dormant intracellular tissue cysts (pseudocysts) that harbour slow-growing bradyzoites (30). Brain, skeletal and heart muscles appear to be the most common sites of the bradyzoites (5). Reactivation of the cysts with rupture of the host cell may happen due to primary or acquired defect in the T-cell mediated immunity, for example in HIV infection, and may cause severe disease (5).

Populations of the parasite are mainly differentiated in three genetic clonal archetypal lineages with different virulence, host effect and geographical distribution: types I, II and III. Additionally, these are divided into several recombinant subtypes (31). Type II is the most common in Europe and North America. Type I and several nonarchetypal *T. gondii* strains are more often observed in South America causing more severe disease (32, 33). Type III is most commonly seen in animals (32).

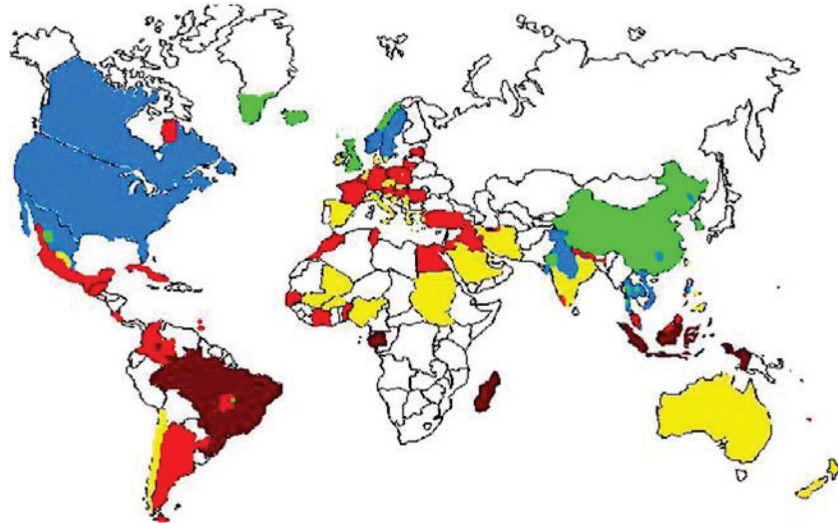
3.3 Epidemiology

The frequency of primary maternal toxoplasma infection depends on the proportion of seronegative pregnant women who are susceptible to infection and on the prevailing infection risk (34). After primary infection, lifelong immunity develops, which can be demonstrated by the presence of toxoplasma immunoglobulin G (IgG). The prevalence of toxoplasma IgG in women of fertile age indicates indirectly the general susceptibility to infection in the pregnant population as a whole. Knowledge of the prevalence is important because preventive guidelines are most often based on this information.

The parasite *T. gondii* exists worldwide with considerable variations between different geographical areas and between different populations within one area (35). The worldwide prevalence of toxoplasma IgG antibody among the pregnant population or among females of reproductive age ranges from 1 to 84% (Figure 2) (36). The highest prevalence has been reported in Africa, South East Asia and Central America with the lowest in Japan, Korea and the northern parts of Scandinavia (36).

Figure 2. Global status of *Toxoplasma gondii* seroprevalence among pregnant women or women of childbearing age (36)**.** Used with permission from Elsevier publisher Ltd: [Injt j Parasitol.], Pappas et al. 2009[36.]

Dark red equals prevalence above 60%, light red equals 40–60%, yellow 20–40%, blue 10–20% and green equals prevalence <10%. White equals unknown.



In general, the toxoplasma IgG prevalence increases with age and tends to be higher in moist, warm areas and lower in cold regions (23). In addition to climate, the differences in prevalence may be ascribed to variations in hygiene and eating habits (37, 38). French cuisine has been thought to cause the relatively high prevalence of toxoplasma IgG among the French pregnant population.

During the last decades, a reduction in the prevalence of IgG antibodies has been observed in Europe, coinciding with the increased industrialization of farming (39-41). In France, the prevalence of toxoplasma IgG among pregnant women has declined substantially, in the last 30 years, from 80% to 43% (39).

3.3.1 Prevalence and incidence in the Nordic countries

The first study on prevalence in the north (Sweden) was published in the 1950s reporting approximately half of the pregnant women to be toxoplasma IgG positive (42). The prevalence of toxoplasma IgG in our neighbouring countries is as follows: Sweden in 2000 14% in Stockholm and 25.5% in Skåne (43), Iceland in 2006 9.3% (women and men <39 years) (40), Denmark in 1995 27% (44) and Finland in 1992 20% (45).

In Norway, Harboe reported the prevalence of positive dye test titre in blood donors in 1952 to be 8.6% and Vaage and Midtvedt reported the prevalence among naval recruits to be 13.4-

34.7% in 1975 (46, 47). A study conducted in 1978 showed a significantly higher seroprevalence of toxoplasma IgG antibodies (47-52%) among children with visual and mental disabilities (48).

The information on the Norwegian prevalence among pregnant women originates from studies conducted in the seventies and early nineties and was found to be 12.6% and 10.9%, respectively (23, 49). In 1992 and 1993, the prevalence within the population of women living in the Oslo area was higher (13.2%) compared to northern Norway (6.7%) (23). In 2009 Bjerke found a higher prevalence of toxoplasma IgG among pregnant Pakistani immigrants (17%) living in Oslo (50). In Sweden, a tendency of reduction in toxoplasma IgG prevalence has been shown in identical age groups of non-immigrant Swedes, from 47.7% in 1957 to 11% in 2006 (40, 42). This change may be attributed to several factors: a change in the animal hold, better meat control, increased consumption of instant or ready cooked food, home freezers for food storage, and education and screening programs for pregnant women (51-53). The reduction has not been observed among the immigrant population (42). In addition to potential climate changes and increased immigration, great changes in diet and travelling habits have taken place in the Norwegian population during the last fifty years of economic growth (http://www.yr.no/sted/Norge/Østlandet/klima.html) (54, 55) .

The incidence of maternal primary toxoplasma infection varies from country to country, depending on the prevailing infection risk. In Norway, the incidence of maternal primary infection was calculated to be 2 per 1000 in the seventies, and 1.7 per 1 000 pregnancies in 1994 (22, 24). The incidence was 4.6 per 1000 in the capital city Oslo (24). In Sweden, the risk of primary infection among susceptible pregnant women is estimated to be 0.51 per 1000 pregnancies, in Finland 2.4 per 1000 and in Denmark 2.7 per 1000 (44, 45, 56).

3.4 Transmission

3.4.1 Acquired toxoplasmosis

Humans are most commonly infected by ingestion of contaminated food or water containing oocysts or from tissue cysts (bradyzoites) in undercooked or raw meat (Figure 1) (37, 38). In the beginning of the nineties, a prospective case-control study examining risk factors for being infected with toxoplasma in pregnancy was conducted (37). The strongest risk factors were eating raw mutton (OR 11.4), pork (OR 3.4) or raw minced meat products (OR 4.1), findings supported in the literature (38, 57-59). In addition, cleaning the cat litter box (OR 5.5) and poor kitchen hygiene (OR 7.3) were associated with maternal toxoplasma infection. Travel to countries outside of Scandinavia was not an independent risk factor when

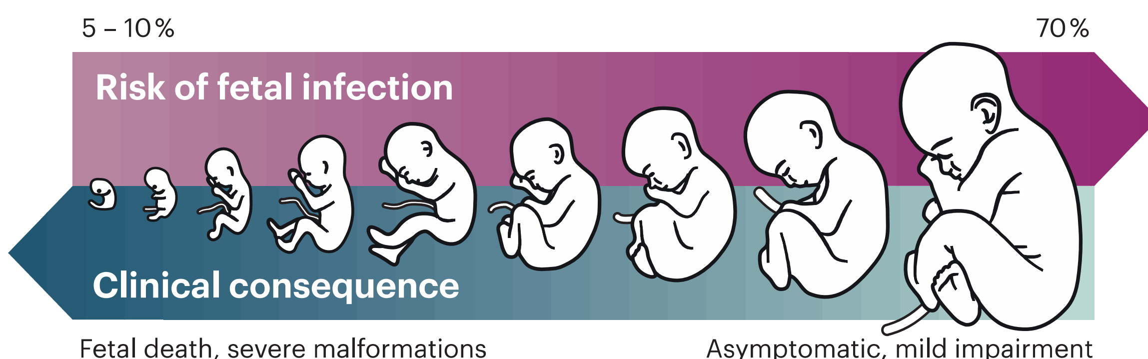
controlling for the other risk factors. In the review by Petersen et al. published in 2009, they described a substantial proportion of infected women without any known risk behaviour (60). Furthermore, the parasite may be transmitted through blood transfusion, by organ transplantation or direct inoculation of parasites by laboratory accidents (5, 61, 62).

After primary infection, immunity develops, as demonstrated by toxoplasma IgG antibodies. Thereafter, the person has a lifelong latent infection. Infection acquired before pregnancy does not affect the foetus because the foetus is protected by the maternal IgG antibodies (63).

3.4.2 Congenital transmission

Congenital transmission of the parasite was the first mode of transmission to be recognized (8). Isolation of parasites from placenta and infected neonates suggested that the parasite was transferred from the maternal bloodstream via the placenta to the foetus (5). Several factors might influence the degree of the foetal infection; parasite strain, virulence, parasite inoculum number, gestational age (GA) at infection and immune competence of the mother and the foetus (5). Evidence indicates that foetal infection occurs in the early phase of maternal infection, during her parasitemia, i.e. most commonly during the first weeks of acute infection before the development of maternal antibodies and before the appearance of clinical signs and symptoms (5). Replication of the parasites in the placenta may cause a delay between maternal and foetal infection (64). The earlier the foetus is infected, the more serious is the outcome (Figure 3) (5). Women with the highest risk of delivering a child with congenital infection appear to be those infected between the 10th and 24th gestational weeks (GW), in particular, before GW 16 (65). The overall transmission rate in pregnancy is 23-30%, but is lower in first trimester (<10%) and up to70 % at term (24, 39, 66).

Figure 3. Risk of foetal infection and clinical implications for the foetus throughout pregnancy



3.5 Clinical manifestations

Acquired toxoplasma infection is usually asymptomatic in immunocompetent humans. However, toxoplasmosis may present as nonspecific flulike symptoms, fatigue or gastroenteritis and with signs like lymphadenopathy or rash (5, 67). Cervical lymph nodes are the nodes most commonly involved, and the liver and spleen may be affected (67-69). Chorioretinitis and opticus neuritis are reported in immunocompetent individuals, in particular, in South America where the more pathogenic toxoplasma type I and nonarchetypal strains are dominating (70). In immune-compromised individuals, the parasite may cause a fatal disease, most commonly encephalitis (71, 72).

Transmission to the foetus causes a wide spectrum of clinical features, ranging from asymptomatic or mild visual disturbances to spontaneous abortion, foetal death and severe brain damage (5, 24, 66). Foetal infection during the first trimester will often result in miscarriage or severe malformations commonly resulting in termination of the pregnancy. The classic tetrad of symptoms and findings of the neonate when infected as foetus early in pregnancy are chorioretinitis, hydrocephaly, intracranial calcifications and convulsions (9). New-borns with a clinically apparent disease have a poor prognosis with high mortality (73). Diebler et al. observed a clear relationship between the cerebral lesions found on CT (calcifications, cysts and hydrocephaly) and neurologic signs, and gestational time of maternal infection (74). Intracranial calcifications may be found scattered through the brain, detected during ultrasound examinations or CT scans. The latter may also detect changes not shown by ultrasound (5). In addition, MR may be used in diagnosing congenital changes (75, 76). Chorioretinitis is diagnosed through fundoscopy. If primary maternal infection occurs in the third trimester, the outcome is often an asymptomatic infant with subclinical infection or mild impairment (77-79). Nevertheless, about 30% of the infants will develop chorioretinitis during childhood or adolescence and 10-34% will have some degree of neurological sequelae (77, 78, 80-82). In recent years, researchers have hypothesized that infection with *T. gondii* may cause psychiatric disorders like schizophrenia and self-directed violence (83, 84).

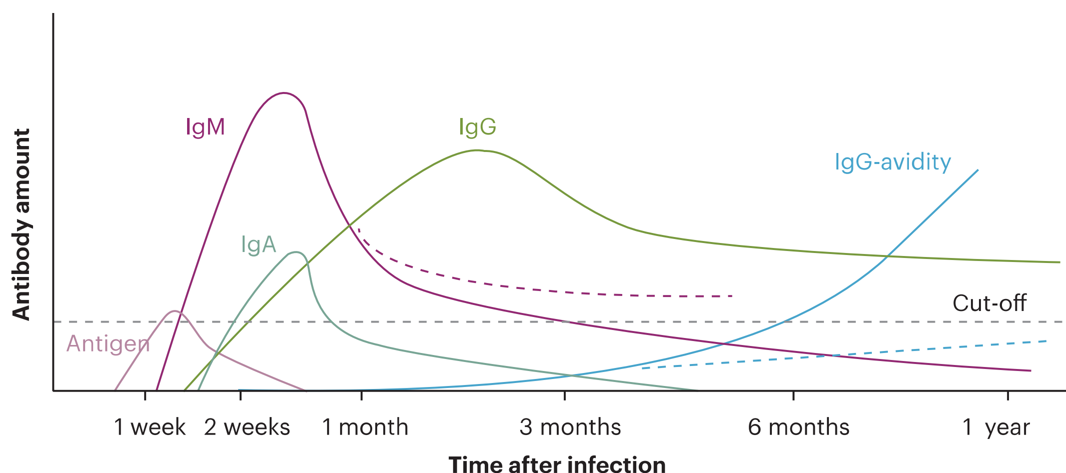
3.6 Diagnosis

Over the years, several tests to detect maternal and foetal toxoplasma infection have been developed. Infection may be detected indirectly by demonstration of antibodies or directly by demonstration of parasites (tachyzoites or bradyzoites) in placental or foetal tissue or by demonstration of parasitic nucleic acid by polymerase change reaction (PCR) in blood, tissue or cerebrospinal fluid. Attempts to isolate the parasite are most often performed by injection

of possibly infected material into laboratory mice or by inoculation into tissue culture preparations (most often embryonic fibroblasts) (5, 85-87).

Maternal infection is detected serologically through demonstration of *T. gondii* antibodies; positive IgG and IgM and low IgG avidity indicate primary infection and positive IgG, negative IgM and high IgG avidity latent infection (Figure 4) (25, 88, 89). The diagnosis of foetal infection is based on a demonstration of the parasite or parasite DNA in amniotic fluid or foetal blood. Infection is further verified through PCR and serology in the neonate after birth and serology follow-up during the first year of life (90). Because maternal IgG is transferred to the foetus, only IgA and IgM are used to evaluate congenital infection in the neonate.

Figure 4. The development of toxoplasma antibodies after acquisition of infection



3.6.1 Antibody-tests

Several serologic tests are available for the measurement of antibodies against *T. gondii* and a panel of tests is often used by the reference laboratories to determine whether the toxoplasma infection is latent or primary. The initial evaluation is to analyse toxoplasma IgG, IgM antibodies and IgG avidity. If toxoplasma infection is suspected in pregnancy, a new sample, ideally after three weeks, should be analysed for immunological changes and confirmation of the result (91). IgG seroconversion and at least a two-fold rise in IgG amount indicates primary infection. IgG and IgM positivity and low IgG avidity indicate the possibility of a primary infection (Figure 4).

3.6.1.1 Sabin-Feldman dye test

The first test developed to detect toxoplasma infection was the Sabin-Feldman dye test (14). Living parasites incubated in fresh non-infected serum swell and stain deep blue when indicator fluid is added. If the parasites are exposed to antibody containing serum, it will not stain because antibodies bind to and kill the parasites due to complement mediated lysis of the cell membrane (5). The titre is the inverse value of serum dilution at which 50% of the parasites are killed, expressed in IU/ml. In previously infected individuals the titres normally range from 2 to 200 IU/ml, while in patients with primary infection the titres may get high (> 300 IU/ml). However, this threshold is neither sufficiently sensitive nor specific to diagnose primary infection (89, 92). The test is still considered as the gold standard for IgG antibody detection, but the method is tedious and expensive and is not provided for routine use any longer.

3.6.1.2 Agglutination tests

These tests use either whole parasites preserved in formalin [direct agglutination (DA) assay] or latex particles or erythrocytes coated with sonicated tachyzoites (indirect agglutination assay) as antigen (93).

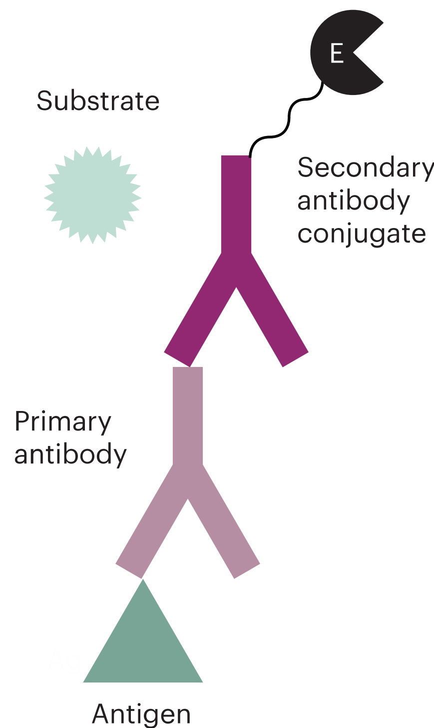
Differential agglutination test (also known as the "AC/HS test") uses two antigen preparations that express antigenic determinants found early in acute infection (AC antigen) or in the later stages of infection (HS). Because antibodies produced early in the infection react differently to the antigens from the antibodies produced later, the test makes it possible to differentiate between recent and chronic infection (94). This test has proved useful to differentiate latent from primary infections but is best used in combination with a panel of other tests.

3.6.1.3 The ELISA and CMIA-assays

The ELISA (Enzyme-linked immunosorbent assay) /MEIA (microparticle enzyme immunoassay) / CMIA (chemiluminiscent microparticle immunoassay) -technology has largely replaced other methods in routine laboratories. These methods can detect IgG, IgM and IgA separately. The assays use antigens that either is a lysate of parasites, preparations dominated by the purified membrane protein P30, or antigens produced by recombinant DNA technique (95-97). In the IgG test these antigens are immobilized on a solid surface (Figure 5). Specific antibodies attach to these antigens when the patient's serum is added and unbound antibodies are washed away. Labelled anti-human-IgG-antibodies (conjugate) are added and bind to the attached antibodies. After additional washing, the bound conjugate is detected by

colour/chemiluminiscence when a substrate is added. In the most common IgM test, the patient's antibodies are bound to anti-human-IgM antibodies on the solid phase, and the reaction is detected by a conjugate of enzyme-labelled specific antigen followed by adding the substrate.

Figure 5: The Enzyme Linked Immuno-sorbent Assay or ELISA-test, detecting toxoplasma specific antibodies (http://tube.medchrome.com/2011/11/elisa-test-antibody-detection.html).



3.6.1.4 Immunosorbent agglutination assay (ISAGA)

This method combines the advantages of direct agglutination assay and the ELISA test, is easy to perform and avoids the presence of false positive results due to the rheumatoid factor and unspecific antibodies in serum (98). The test has a high sensitivity and specificity and enables detection of IgM very early in acute infection but also for a longer period after acute infection (5, 99). A variant of the method can be used for detection of specific IgA.

3.6.1.5 The IgG avidity test

The strength of the antibody binding to the antigen (-avidity) increases with time (88, 89, 100). Different tests measure the antigen binding avidity of IgG antibodies (88). The method is most commonly used in order to decide whether the pregnant woman acquired the infection prior to conception or during pregnancy. Avidity cannot give precise information on the duration of the infection, but high avidity clearly indicates the occurrence of infection more

than four months earlier (Figure 4) (89, 100, 101). Low avidity indicates a possible but unconfirmed primary infection since low avidity in some patients may last for several months (Figure 4).

3.6.2 Detection of Toxoplasma gondii by cultivation

3.6.2.1 Mouse inoculation

T. gondii can be isolated through insertion of infected material, such as amniotic fluid, into mice. Most often this is performed intraperitoneally or subcutaneously. The procedure requires living parasites and must, therefore, be effectuated rapidly after the material is obtained. After 3 to 6 weeks, blood sample from the mouse is analysed for toxoplasma antibodies and the diagnosis is confirmed by the presence of tissue cysts in the brain (5). The PCR-test has now in most laboratories replaced mouse inoculation because of higher sensitivity and a rapid result (102).

3.6.2.2 Cell cultures

The parasite may also be cultured *in vitro* in cell culture. Infected material is injected in to tissue culture preparations, most often embryonal fibroblasts. Tissue culture isolation is rapid and enables diagnose in about one week. Infected cells can be detected with fluorescence microscopy (5). However, this is a less sensitive method than mouse inoculation (87, 103).

3.6.3 Detection of Toxoplasma gondii-DNA

The presence of *T. gondii* in a specimen can be confirmed by detection of the parasite DNA with the PCR method (102, 104, 105). In 1990, Grover et al. described the efficacy of the PCR method for rapid diagnosis of congenital infection (106). PCR has been performed on amniotic fluid after amniocentesis with sensitivity 59 - 97% and specificity 94 -100% depending on gestation, method and laboratory (102, 105, 107-109). In the study by Jenum et al., the sensitivity was 59% and the specificity 94 % (102). Conventionally, the B1 gene of the parasite has been the target of PCR, but recently the 529-bp fragment has been shown to be more informative (108, 110, 111). The sensitivity has been found to be highest in the second trimester and lowest in the first trimester (107, 112).

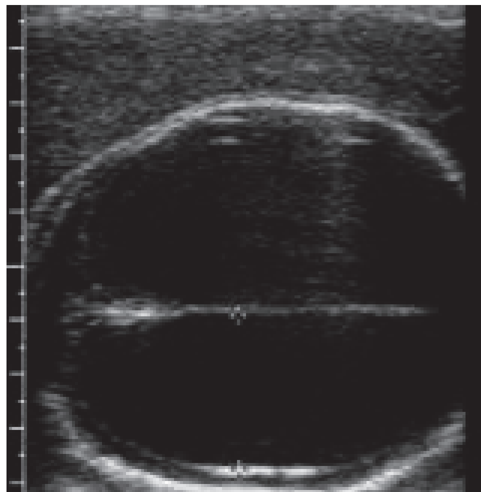
3.6.4 Ultrasound

Foetal ultrasound has a significant place in diagnosis and follow-up of possible antenatal toxoplasma infection, both for detecting foetal abnormalities and for performing amniocentesis. The classical findings are intracerebral calcifications, hydrocephaly, thickened

and echo dense placenta, hepatomegaly, splenomegaly and, less often, microcephaly, pericardial fluid and growth restriction (113). In the study by Hohlfeld et al. 32/89 (36%), infected foetuses had ultrasound detected anomalies (113).

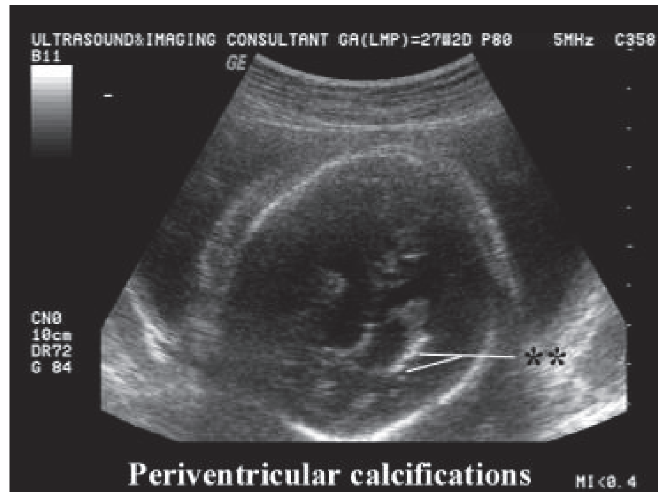
Figure 6. Classical ultrasound findings in foetal toxoplasma infection

Hydrocephaly



www.fetalsono.com

Intracerebral calcifications



www.fetalultrasound.com

3.6.5 Amniocentesis

During the last 50 years, amniocentesis has been available as a method of detecting genetic defects in the foetus (114). In the last 20-30 years, amniocentesis has been the method of choice for verifying foetal toxoplasma infections (105, 115). The procedure is performed under ultrasound guidance with needle gauge of 18-20. There have been speculations concerning needle gauge and the risk of procedure-related complications. In one small randomized controlled trial comparing needle gauge 22 with gauge 20, they did not report any difference in complications (116). The amount of fluid obtained diverges from 1-2 ml to 20 ml in different trials. The most serious complication after amniocentesis is miscarriage. GW and operator skills have an impact on the procedure-related risk, which is estimated to be 0.5-1.0% (117, 118). In recently published studies the risk of miscarriage after the procedure is reported to be similar to the background population (119); however, it is advised not to perform amniocentesis until GW 15+0 (117).

3.7 Treatment

The treatment effect of commonly used antiphrastic drugs on the parasite *T. gondii* has been shown in several *in vivo* and *in vitro* studies (10-12, 120-123).

An acquired infection in immunocompetent humans usually has a benign character and specific treatment is rarely indicated; nevertheless, one exception is a primary infection in pregnancy. Pyrimethamine, sulphonamide and spiramycin are the most common drugs used for treating acquired or congenital infection with *T. gondii*. Primarily these drugs slow down the multiplication of tachyzoites (124).

Since the beginning of 1950s, the antimalarial drug pyrimethamine in combination with sulphonamides have been the drugs of choice when antenatal or neonatal infection is verified (10-12). However, these drugs do not eliminate tissue cysts, unlike azithromycin and atovaquone, which have been shown to break down the cysts (125). Pyrimethamine penetrates the blood-brain and the retina barriers and concentrates in the brain and retinal tissue (126). The drug acts as a folic acid antagonist and the main adverse effect is, therefore, thrombocytopenia and leukopenia. Folic acid is, therefore, given as a supplement to the treatment and the hematologic profile is regularly controlled (127). Pyrimethamine-sulphadiazine may be used in second and third trimester in order to prevent mother-to-child transmission; however, spiramycin, azithromycin or other macrolides are more commonly used in this situation (128).

The macrolide spiramycin kills free and intracellular parasites but does not penetrate the blood-brain barrier to a sufficient extent and is, therefore, seldom used as a single treatment (129). Spiramycin has failed to cure toxoplasma encephalitis in immunocompromised patients (130). The drug accumulates in the placenta with concentrations up to five times higher than in maternal serum. Spiramycin crosses the placental barrier, but foetal concentration is lower than the maternal (131). No teratogenic effect is reported (http://www.relis.no). Spiramycin is usually given to prevent or reduce mother-to-child transmission.

In the last 20 years, azithromycin has been used to a great extent in Norway for preventing mother-to-child transference. The drug has a high tissue penetration and a high placental concentration is obtained (132). Azithromycin has a long half-life and only one dose is required three days a week (spiramycin nine tablets every day). There have been concerns about teratogenicity in the first trimester, though such observations from a Swedish study have not been replicated (133, 134). The treatment protocol in Norway is now to prescribe

azithromycin or spiramycin until the infection is verified by amniocentesis and toxoplasma PCR. In cases of infection after the 18th GW, the treatment is changed to pyrimethamine-sulphadiazine (http://legeforeningen.no/Fagmed/Norsk-gynekologisk-forening/Veiledere/Veileder-i-fodselshjelp-2014/Bakterielle-infeksjoner-hos-gravide/Toksoplasmose).

Alternative drugs are clindamycin and trimethoprim-sulfamethoxazole. These drugs are not elaborated here since they are seldom used in pregnancy.

The question on whether antiparasitic treatment prevents transmission and neonatal complications has been discussed during the last 50 years. Different approaches have been examined to establish the benefit of treatment (135). Daffos et al. and Hohlfeld et al. reported that pyrimethamine-sulfadiazine is more effective than spiramycin in preventing severe foetal infection (65, 105). This was supported by Couvreur et al. in 1993 (122). In 1994, Foulon et al. studied 144 pregnant women and their offspring and found a significant reduction of sequela in the infants after maternal treatment; however, they did not verify Desmonts' findings of a reduction in foetal transmission after spiramycin treatment (128, 136). In a study on rhesus monkeys, treatment with pyrimethamine-sulfadiazine prevented further foetal infection if started immediately after verified antenatal infection (137). In a European multicentre study, they did not find a decline in mother-to-child infection by treatment, but they observed less severe clinical manifestations in the treated group (80). Interestingly, they found no difference between spiramycin monotherapy and pyrimethamine-sulfadiazine treatment in terms of reducing serious manifestations in the offspring. Gratzl et al. examined the concentration of spiramycin in maternal and foetal serum and amniotic fluid (138). They found that none of the concentrations measured were sufficient to inhibit the growth of the parasite (based on concentrations reported in *in vitro* studies) (138). They explained the variability in maternal and foetal drug concentrations by individual differences in several pharmacokinetic parameters and a possible lack of compliance.

It seems obvious that treatment after mother-to-child transmission will not reduce the transmission rate if not started immediately after infection, but the treatment may influence and modify the severity of the foetal infection. Today, most researchers assume that the foetus is infected very soon after the maternal infection, and thus for the treatment to have any effect on the transmission rate, it has to be initiated immediately after the maternal acquisition, which rarely happens (139).

Because of drug resistance, side effects and suboptimal effect in preventing congenital toxoplasma infection, research on new drugs, natural drugs and vaccines are ongoing (140-142).

3.8 Screening for Toxoplasma antibodies among pregnant women

Screening policies to prevent congenital toxoplasma infection differ between countries, from no prenatal screening (in Scandinavia, the United Kingdom and the United States) to monthly screening as for example in France or bimonthly screening as in Austria. The differences in the prevalence of toxoplasma IgG among pregnant women contribute to these differences. During the 1980s, cheap and reliable laboratory methods were introduced, making large-scale testing possible (143-146). Therefore, in 1992 the Norwegian National Institute of Public Health conducted a large cohort study exploring the epidemiology of antenatal toxoplasma infection in Norway. The overall toxoplasma IgG prevalence among pregnant women was 10.9% and the incidence of maternal primary infection was calculated to be 0.17% (23, 24). A cost-benefit analysis from 1992 concluded that the incidence of antenatal toxoplasma infection exceeding 0.1-0.15% would justify screening (147); however, a national screening programme on toxoplasma infection during pregnancy in Norway was declined (148). This was mainly due to a lack of sufficient scientific evidence of the benefits of such a programme, and more specifically the uncertainty concerning the effect of treatment.

The national neonatal screening programme for congenital toxoplasmosis in Denmark (1977-2007) was discontinued after evidence of no treatment effect neonatally (149).

3.9 Present diagnostic challenges

New data on the prevalence of toxoplasma-specific IgG antibody in pregnant women in Norway are lacking, and the most recent study on this issue in Norway was conducted 20 years ago. Despite the fact that antenatal toxoplasma screening has been discouraged in Norway, toxoplasma serology is frequently performed on request from the patient or her healthcare provider and is often obtained at the end of the first trimester. The presence of toxoplasma IgM, IgG and low IgG avidity indicates possible maternal infection, but positive toxoplasma IgM and low IgG avidity may persist for months and even years. This leaves the physician with the difficult task of evaluating whether the women is infected prior to or during pregnancy, and whether amniocentesis should be recommended to further clarify if foetal infection has occurred and whether antiparasitic treatment is necessary. Few studies have addressed the impact of IgG avidity in pregnancy and use of amniocentesis in a

population with a low prevalence of *T. gondii* infection in which no systematic screening is performed.

4 Aims of the studies

The overall aim of this thesis was to update the knowledge on the prevalence of toxoplasma IgG among pregnant women in Norway and to explore the diagnostic and follow-up of suspected toxoplasma infection during pregnancy among our patients in Southeast Norway.

Specific aims

Paper I: To estimate the toxoplasma IgG prevalence among pregnant women in Norway and to compare the results with the prevalence found in Norwegian studies conducted in 1974 and 1992.

Paper II: To examine the development of the toxoplasma IgG avidity in pregnant women with the focus on duration of low avidity.

Paper III: To evaluate the use of amniocentesis to diagnose foetal toxoplasma infection with the aim of determining whether amniocentesis is performed on the correct patients and whether the procedure is safe for this indication.

Hypothesis

Since a reduction in the toxoplasma IgG prevalence rates is observed among pregnant women in Europe, we hypothesized that the prevalence among pregnant women in Norway has declined. However, because there is an increase in the risk factors in the population, an increase in prevalence might be possible.

In the second part of the project, we hypothesized that the serology testing and interpretation of primary toxoplasma infection in pregnancy in Norway is not optimal, leading to unnecessary amniocenteses in order to diagnose foetal toxoplasma infection.
Because less amniotic fluid is required for toxoplasma-PCR compared to genetic testing, we expect a low complication rate.

5 Material and methods

The data in this thesis originates from pregnant women in Norway who belong to one of two different cohorts:

1. A *prevalence study* using 2000 sera obtained from a cross-sectional study of pregnant women in Buskerud and Sør-Trøndelag (paper I).
2. A *retrospective cohort study* of data from all women with amniocentesis on the indication suspected toxoplasma infection (papers II and III).

5.1 Study population

5.1.1 Paper I

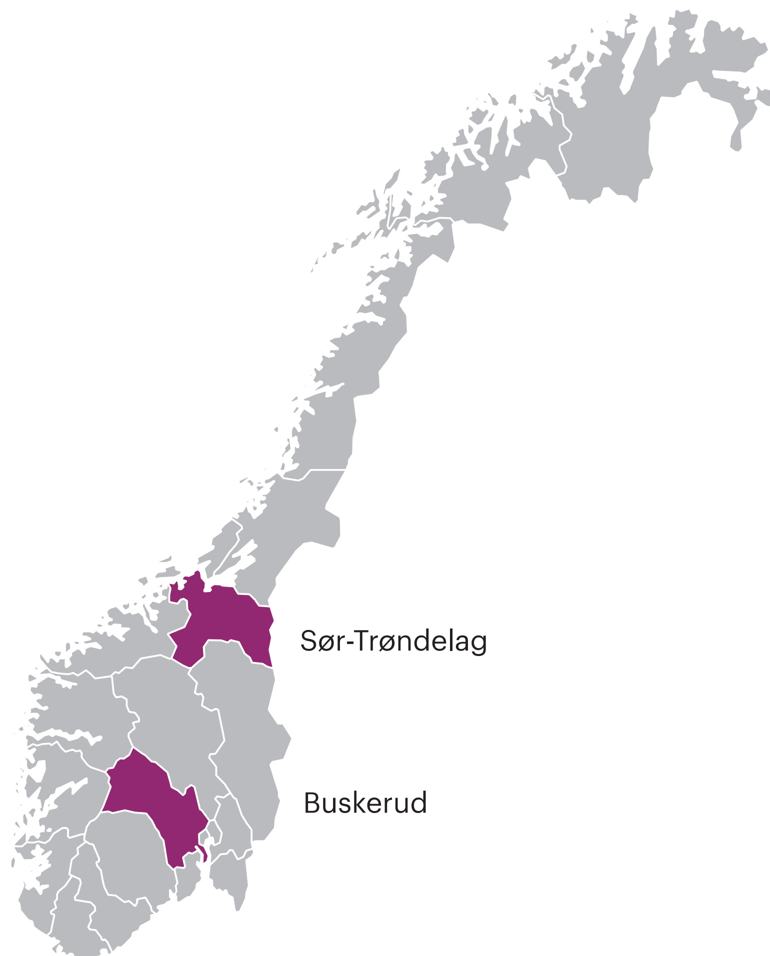
The prevalence study was a sub-study of the cross-sectional project «Immunity in the Norwegian population», initiated by the Norwegian Institute of Public Health in 2010 where the prevalence of rubella, cytomegalovirus and parvovirus B19 in the pregnant population was studied (150).

During the period from August 2010 to May 2011, 2000 individual serum samples were collected consecutively from pregnant women in Buskerud county in Southeast-Norway (999 samples) and in Sør-Trøndelag county in Mid-Norway (1001 samples) during their first antenatal visit to a primary health care facility. The samples were sent to the local hospital laboratories as part of the routine voluntary screening programme for HIV, rubella and syphilis. These laboratories cover two different parts of Norway (Figure 7), each covering 4.7% and 6.2% of the total number of births in Norway in 2010 (Årstabeller for medisinsk fødselsregister 2010. Fødsler i Norge. www.fhi.no: Folkehelseinstituttet).

For each enrolled sample, data on maternal age, county location and sampling date was collected; the samples were anonymized and sent to the Norwegian Institute of Public Health and stored at -20°C until analyses were performed according to national research bio-bank regulations (https://lovdata.no/dokument/NL/lov/2003-02-21-12).

In the present study, the participants were included if the serum sample had sufficient volume for analysis (1922 out of 2000).

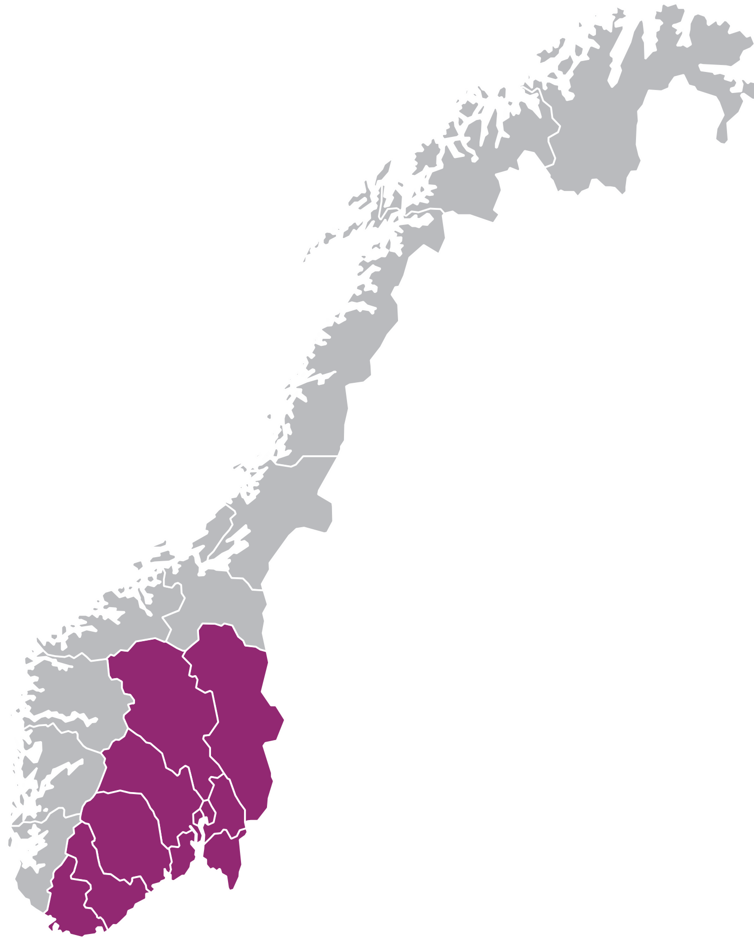
Figure 7. Buskerud and Sør-Trøndelag counties



5.1.2 Papers II and III

In the retrospective study we included all women who underwent amniocentesis as part of the diagnostic routine at Oslo University Hospital due to serologic signs of possible toxoplasma acquisition in pregnancy during a 21 years period from 1 September 1992 to 31 December 2013. The women were mainly referred from primary healthcare centres in the Southeast Health Region (Figure 8), which constitutes approximately half of the Norwegian pregnant population. The study population originally included four women pregnant with twins. In three of these twin pairs with separate amniotic sacks, amniocentesis was performed on both twins. The twin pregnancies were excluded from the analysis in paper III because of the possibility of increased risk of complications by amniocentesis (151).

Figure 8. The Southeast Health Region in Norway



Information on the women was obtained from clinical hospital records, laboratory records and pregnancy charts. Neonatal and infant data were obtained from the maternal records. We did not have access to the paediatric files and additional information was, therefore, collected by a short questionnaire sent to 40 women whose information on gestational age at birth and birth weight was incomplete in the records (Appendix).

Maternal toxoplasma serology was labelled as first and second serology in pregnancy (most often three weeks later than the first for confirmation and evaluation of immunologic changes) and third serology at birth.

GA was estimated according to ultrasound assessment obtained at 17-19 GW or, if not available, from the first day of the last menstrual period. Time of seroconversion was mapped as the GA of the first positive toxoplasma antibody sample because the time point of infection was unknown in most patients.

Amniocentesis under ultrasound guidance was performed at Oslo University Hospital by experienced obstetricians until 1999 and, thereafter, by trained sub-specialists in foetal medicine at the Section of Fetal Medicine at Oslo University Hospital. The amniotic fluid was brought directly to the microbiological laboratory stored at room temperature.

In paper II, the material was collected from the above-mentioned cohort. Only women with initial low toxoplasma IgG avidity and follow-up sera sampled over a period of three months or longer, were included. A total of 176 women provided 542 serum samples, on average three samples per woman.

5.2 Laboratory analyses

5.2.1 Paper I

The specimens were examined for toxoplasma-specific IgG antibodies using an indirect ELISA test (Bio-Rad, Marnes-la-Coquette, France), the same method that was used in the national prevalence study in 1992-93 (23). The analyses were performed on the BEST 2000® analysing instrument (Werfern Group, Biokit, Barcelona, Spain), allowing a small volume of each serum to be analysed (15 µl). The samples were analysed at the certified laboratory at the Department of Laboratory Medicine, Section of Medical Microbiology, Vestre Viken Hospital Trust, during the autumn of 2012 and the winter of 2013. The results of the analyses were validated against independent controls included in each run. Antibody levels were quantified and expressed as international units per ml (IU/ml) according to WHO standards and interpreted according to the manufacturer's recommendations. A value of <4 IU/ml was regarded as negative, reflecting susceptibility to infection during pregnancy; 4 - <8 IU/ml was interpreted as borderline, and ≥8 IU/ml was regarded as positive, indicating previous or on-going *T. gondii* infection. We also estimated the prevalence of toxoplasma IgG according to a cut-off value of 6 IU/ml for positive IgG because this was the cut-off used in the previous study by Jenum et al. (23). To explore the possible variation in prevalence, the result was additionally calculated with a cut-off of 10 IU/ml.

The diagnostic sensitivity and specificity of the *T. gondii* IgG ELISA test have been shown to be 96.8% and 100%, respectively (95).

5.2.2 Papers II and III

All serum samples were either analysed at the Norwegian Institute of Public Health (before 2002) or at the Toxoplasma Reference Laboratory at Oslo University Hospital (established in 2002). During the course of the study period, the test routine for toxoplasma antibodies was changed several times (Table 5, Appendix). The following tests were used:

Dye test detecting total antibodies (14).

Agglutination tests: Toxo-Screen DA IgG and Toxo-ISAGA IgM (bioMérieux, Marcy l´Etoile, France).

Indirect enzyme-immunoassay (EIA) (Platelia Toxo IgG and Toxo IgM, Diagnostic Pasteur/Bio-Rad, Marnes-la-Coquette, France), microparticle enzyme immunoassay (MEIA) (Axsym, Abbott, Wiesbaden, Germany) or chemiluminescence microparticle assay (CMIA) (Architect, Abbott, Wiesbaden, Germany).

Until June 2005, the avidity method was performed as previously described using an in-house method based on the Platelia Toxo IgG assay and the avidity results were expressed as the percentage of antibodies resistant to elution by urea (89). Thereafter, the commercially available Platelia IgG avidity test was used (Bio-Rad). The results were expressed as an avidity index (AI). The results were interpreted according to a previous publication or the manufacturer's recommendations and described as low, borderline or high avidity (89). Values greater than 20% and AIs greater than 0.5 were considered as high avidity and to be indicative of latent infection, values of 20-15% and AIs of 0.5-0.4 indicated borderline avidity and values of less than 15% and AIs of less than 0.4 indicated low avidity and possible primary *T. gondii* infection.

A significant antibody increase was defined as at least a two-fold increase in IgG antibody level expressed as IU/ml or as a two-titre step increase. A significant increase in IgG avidity was defined as an increase of greater than 5% or 0.1, depending on the test used.

Maternal toxoplasma infection was confirmed by seroconversion of the toxoplasma IgG during pregnancy or by an increase in IgG-antibodies in serum pairs, most often with a three-week interval. The women were divided into three groups according to their toxoplasma serological profile: (i) IgG seroconversion, (ii) significant IgG antibody increase and (iii) IgM positivity and low IgG avidity (dye test titre >300 IU/ml was used before the IgG avidity test was established in1996).

The women were categorised into three groups according to suspected time of infection based on maternal and neonatal serology: (i) infected before pregnancy, (ii) possibly infected during pregnancy and (iii) certainly infected during pregnancy. The evaluation was performed retrospectively and independently by P.A. Jenum and B. Stray-Pedersen, both with expert knowledge in toxoplasma diagnostics and infection in pregnancy. Consensus was reached for interpretation of the results.

In paper II the women were further subdivided into groups according to avidity change: IgG avidity change from low to high, a significant increase in IgG avidity below the high threshold, and stable low IgG avidity. The duration of low avidity was calculated from the time of the first sample with a valid IgG avidity value until the final sample with low avidity.

The presence of congenital infection was confirmed by at least one of the following criterions: (i) positive toxoplasma PCR in amniotic fluid or neonatal cord blood, (ii) positive mouse inoculation of amniotic fluid or cord blood, (iii) positivity for toxoplasma IgM or immunoglobulin A in postnatal serum, or (iv) toxoplasma IgG persisting in the infant at 12 months after birth (90).

Mouse inoculation was performed until 2002 as previously described and PCR analysis based on the toxoplasma B1-gene was introduced as a diagnostic indicator of congenital toxoplasma infection in 1992; both methods are previously described (102). From 2004 the PCR method was changed to real-time PCR of the bp529 gene (110).

5.3 Statistics

5.3.1 Paper I

The IgG-status was analysed, both as a continuous variable (IU/ml) and as a categorical variable divided into IgG-status negative, borderline and positive.
The presence of toxoplasma IgG was estimated by a period prevalence with 95% confidence interval. Continuous variables were described by the median with quartiles and ranges. Maternal age was analysed, both as a continuous and as a categorical variable divided into six groups with a five-year interval (≤19, 20-24, 25-29, 30-34, 35-39 and ≥ 40 years old) in order to simplify the comparison with previously published results (23). The frequency of toxoplasma IgG seropositivity was studied by county and the six age groups. Univariate analysis was performed using contingency tables.

The power estimation was performed before the start of the prevalence study based on the prevalence findings of Jenum et al. (23). The prevalence of toxoplasma IgG seropositivity in pregnant women in Norway was during the nineties estimated to be 10.9% (23). Considering a population of approximately 133 000 women of fertile age in Buskerud and Sør-Trøndelag (www.ssb.no), a type-I error = 5%, power = 80% and precision = 2% (which means accepting a variability in the range of a prevalence between 8.9–12.9%), sera from 926 subjects was needed.

5.3.2 Papers II and III

Continuous variables are described by the mean and standard deviation or the median with quartiles and ranges where appropriate. Categorical variables are presented as numeric values and frequencies. Group comparisons were done using different bivariate analyses depending on the type of variable and normality of distribution. Categorical variables were compared using Pearson`s χ^2 or Fischer's exact test and numerical values were compared using t-test or one-way ANOVA or nonparametrical tests dependent on the normality of the distribution.

For all tests, a p-value < 0.05 was considered to indicate a statistically significant difference.

Data were entered in Microsoft Excel (2010) (paper I) and analysed using IBM SPSS statistics version 20 (version 20.01; IBM Corp., New York, NY, USA). The database for papers II and III was built in EpiInfo (version 3.5.4; CDA, Atlanta, USA). The figure was drawn in Microsoft Excel, IBM SPSS statistics version 20 and in the R project for statistical computing, “R” (version 3.2.2. R Foundation for Statistical Computing, Vienna, Austria).

5.4 Missing data/secondary exclusions

Paper I: The first part of the project only excluded women if their test tube was lacking or did not contain sufficient serum for the toxoplasma IgG-analysis.

Papers II and III: The second part of the project was a retrospective study. The data depended on the mapped information in patient records and laboratory files. For some variables, for example concerning risk factors for toxoplasma infection, maternal education and health and symptoms and signs of toxoplasmosis substantial information were missing in several patients. Particularly for the period 1992-93, several patients’ journals were missing from the journal storage.

Twins (four pairs) were excluded in paper III.

5.5 Ethical aspects and funding

Paper I: The research programme «Immunity in the Norwegian population» was approved by the Regional Committee for Medicine and Health Research Ethics (2009/1322-3). An updated request was sent to the committee, but the present study was considered a part of the main study (2012/203) and was performed without consent from the patients according to the "Act relating to control of communicable diseases" ([Smittevernloven]) § 3-7 concerning the use of anonymized sera (152).

Papers II and III: Our second project was classified as a "quality control project" by the Regional Committee for Medicine and Health Research Ethics (2011/1310/REK.14.09.11). The project and the publications were evaluated and approved by the Board of Patient Safety at Oslo University Hospital (2012/9519.13.06.12). Due to the project classification and long inclusion period, written consent was not needed. Patient information was, therefore, de-identified prior to the analysis and each patient was given a project ID-number. The list of numbers and names was stored separately from the database which was stored on the hospital computer server on a locked password protected area where only the project leader and the research fellow had access. The information was anonymized after analysis and the database will eventually be destroyed.

The research fellow was employed at the Oslo University during the study period. Vestre Viken Hospital Trust funded the toxoplasma IgG analyses in Paper I. No additional funding was obtained.

6 Synopsis and main results of the papers

6.1 Paper I

Findal G, Barlinn R, Sandven I, Stray-Pedersen B, Nordbo SA, Samdal HH, Vainio K, Dudman SG, Jenum PA. **Toxoplasma prevalence among pregnant women in Norway: a cross-sectional study.** APMIS. 2015;123(4):321-5.

Aim: To estimate the toxoplasma IgG prevalence among pregnant women in Norway and to compare the results with the prevalence found in Norwegian studies conducted in 1974 and 1992.

Population: Serum samples were collected from 2000 pregnant women in Buskerud (n = 999) and Sør-Trøndelag counties (n = 1001) in Norway during 2010 and 2011.

Main explanatory variables: County of collected samples and maternal age at sampling.

Outcome measures: Toxoplasma IgG antibody status, mapped as the categories positive, borderline and negative and as exact IgG value (IU/ml)

Main results: The overall prevalence of toxoplasma IgG seropositivity was estimated to be 9.3% (95% CI 8.1-10.7). In Sør-Trøndelag, 10.4% (95% CI 8.6-12.6), and in Buskerud 8.3% (95% CI 6.7-10.2) ($p=0.13$) were IgG seropositive. This did not differ from the prevalence levels found in 1974-75 (12.1%) and 1992-1993 (10.7%) (Figure 9). The prevalence was fairly stable across the fertile age groups. However, we found a higher prevalence among women ≥40 years compared to younger women (OR 2.65, 95% CI 1.30-5.42) (Table 2).

Figure 9. The prevalence of toxoplasma IgG among pregnant women in Norway during the last 40 years

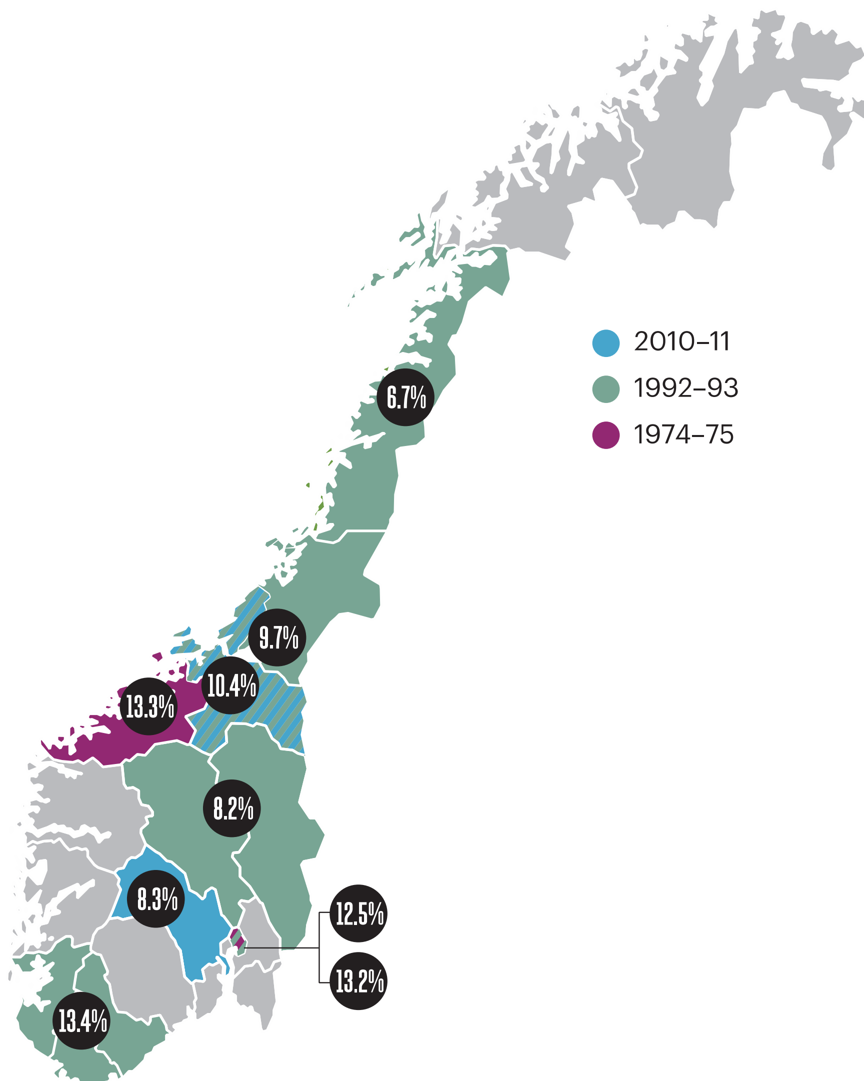


Table 2. Prevalence and IgG values of toxoplasma IgG by age group among 1922 pregnant women in two counties in Norway

Age Groups (years)	Women tested No (%)	*T. gondii* IgG≥8 IU/ml No	IgG prevalence % (95%CI)	Median IgG-value (IU/ml) among IgG positive women Median (Q1,Q3)
≤19	43 (2.2)	5	11.6 (3.9-25.1)	85.3 (37.3, 100)
20-24	281 (14.6)	30	10.7 (7.3-14.9)	26.9 (14.9, 86.0)
25-29	651 (33.9)	59	9.1 (7.0-11.6)	27.9 (14.9,65.7)
30-34	616 (32.0)	52	8.4 (6.4-11.0)	27.5 (20.3, 62.2)
35-39	283 (14.7)	23	8.1 (5.2-11.9)	23.0 (14.7,53.3)
>39	48 (2.5)	10	20.8 (10.5-35.0)	19.2 (16.3, 27.5)
Total	1922	179	9.3 (8.1-10.7)	26.5 (16.4, 65.7)

6.2 Paper II

Findal G, Stray-Pedersen B, Holter EK, Berge T, Jenum PA. **Persistent Low Toxoplasma IgG Avidity Is Common in Pregnancy: Experience from Antenatal Testing in Norway**. PLoS One. 2015;10(12):e0145519.

Aim: To examine the development of the toxoplasma IgG avidity in pregnant women with the focus on duration of low avidity.

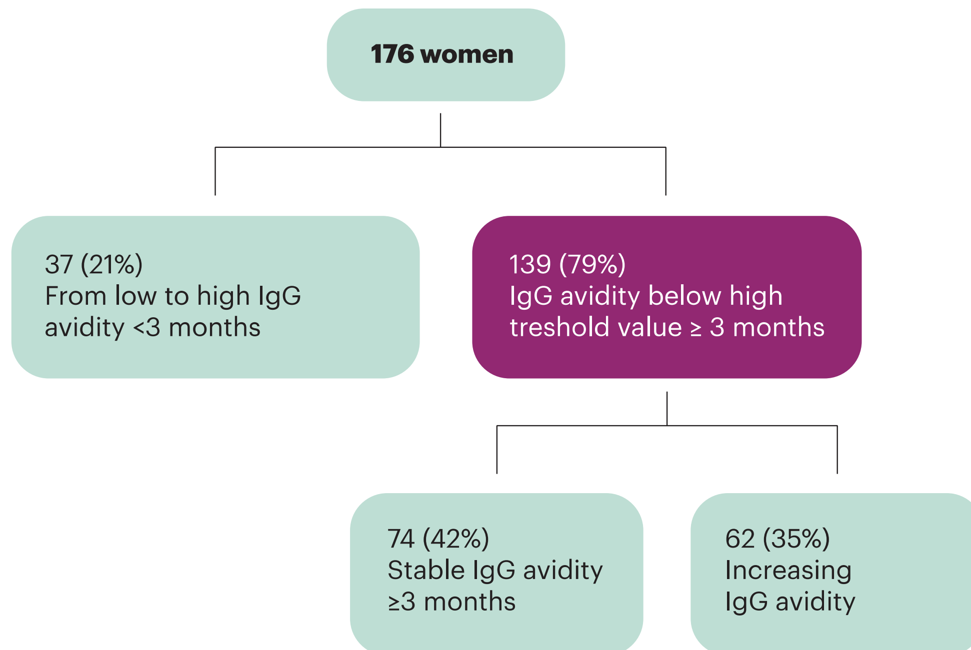
Population: Pregnant women in which amniocentesis was performed due to suspected toxoplasma serology at Oslo University Hospital from 1992 to 2014 with initially low toxoplasma IgG avidity and with IgG avidity measured repeatedly ≥3 months.

Main explanatory variables: GA at IgG avidity analyses, maternal and foetal infection status and maternal serologic group, IgG avidity value as unit (index or %) and further categorized as low, borderline or high according the threshold levels.

Outcome measures: IgG avidity development categorized as increasing from low to high or IgG avidity below the high threshold, which further was dichotomized in to significant increase in IgG avidity below the high threshold value and stable low IgG avidity.

Main results: In 37 (21%) women the toxoplasma IgG avidity increased from low to high in less than three months (Figure 10). In 139 (79%) women the IgG avidity remained below the high threshold for three months or longer. In this group 74 (42%) of them had stable low IgG avidity during the observation period. Median gestational age at first toxoplasma serology was 10.6 weeks (range 4.6–28.7).

Figure 10. IgG avidity changes among pregnant Norwegian women with suspected infection of *T. gondii*



6.3 Paper III

Findal G, Helbig A, Haugen G, Jenum PA, Stray-Pedersen B. **Management of suspected primary Toxoplasma gondii infection in pregnant women in Norway: twenty years of experience of amniocentesis in a low-prevalence population**. Submitted to BMC Pregnancy and Childbirth, 24 August 2016.

Aim: To evaluate the use of amniocentesis in a non-screened low toxoplasma prevalence population in order to determine whether amniocentesis is performed on the correct patients and to assess whether the procedure is safe for this indication.

Population: Pregnant women with singleton pregnancies in which amniocentesis was performed due to suspected toxoplasma serology at Oslo University Hospital from 1992 to 2014 (n=346).

Main explanatory variables: Serologic group, GA at first, second and third serologic toxoplasma sample, GA and year of amniocentesis, amount of amniotic fluid removed, PCR-answer, clinical findings.

Outcome measures: Time of maternal infection, foetal infection, miscarriage

Main results: Maternal infection occurred prior to conception in 173 (50%) of the cases and primary infection was identified in 93 (26.9 %) (Table 3). The serological profiles indicated that 49 (14.2%) women seroconverted, 42 (12.1%) had IgG antibody increase, and 255 (73.7%) women had IgM positivity and low IgG avidity/high dye test titre. In total 15 offspring were infected, of which 14 had positive toxoplasma PCR in the amniotic fluid. In 10 of the infected offspring pathology was present, either at foetal ultrasound, autopsy or clinical examination after birth.

The median GA at the first toxoplasma antibody test during pregnancy was 10.7 GWs (Q_1=8.6, Q_3 =13.4).

Amniocentesis was performed at a median of 16.7 GWs (Q_1=15, Q_3 =22), with a median of 4 ml of amniotic fluid being aspirated (Q_1=3, Q_3=7).

Two miscarriages occurred, both four weeks after the procedure which was performed in GW 13. One of them had a severe foetal toxoplasma infection.

Table 3: Retrospective assessed time of maternal toxoplasma infection according to serologic group

Time of maternal infection	**Seroconversion**	**IgG increase**	**IgM positivity and low IgG avidity**	**n (%)**
Infected before pregnancy	-	-	173	173 (50.0)
Possibly infected during pregnancy	-	-	80	80 (23.1)
Certainly infected during pregnancy	49	42	2	93 (26.9)
Total n (%)	49 (14.2)	42 (12.1)	255 (73.7)	346 (100)

7 Discussion

7.1 Methodological considerations

The purpose of a descriptive study is to describe a population, condition or disease and the existing distributions of the variables (153). Examples of descriptive studies are prevalence studies (cross-sectional studies), surveys, case reports and series (http://www.oxfordjournals.org/our_journals/tropej/online/ce_ch4.pdf).

The quality of a study is often considered in terms of the reliability or precision, and the validity of the findings (154). Precision refers to random error, while validity can be separated into internal and external validity. Internal validity refers to presence of systematic errors or to whether the findings are biased due to the way the data is collected, analysed and interpreted (1). External validity refers to whether the results from the study may be generalized to populations or groups outside the study sample (1). Bias means "being different" and is defined as systematic deviations from the true effects and is present when the association between exposure and outcome is not exclusively the result of the causal effect of exposure on outcome (1).

In this section, first, the overall strengths and limitations of the study methods are discussed. Secondly, the precision and internal and external validity for each part of the study are discussed. The epidemiological terms will be described in the first section (7.1.1) and exemplified in the context of the prevalence study and further exemplified in section 7.1.2 (papers II and III). The prevalence study (paper I) and the retrospective study of the project (papers II and III) will be discussed separately.

7.1.1 Paper I

Sources of error in the estimated prevalence in epidemiological studies may be classified as random or systematic. Random errors lead to loss of precision, whereas systematic errors reduce the validity of the results (154).

Strengths and limitations

<u>Strengths</u>

The strength of the first part of the project includes the chosen design. Observational studies are non-experimental by nature. To estimate prevalence's, an observational study design, more specifically, the cross-sectional design is commonly chosen (155). Cross-sectional studies are regarded as cheap and simple to perform and enable inclusion of large study

populations in a short time (155). They are performed within a defined time period, includes a defined sample population and the condition or main outcome must be clearly defined. We examined the prevalence of toxoplasma IgG antibodies in serum samples with values > 8 IU/ml, which is a well-defined outcome. The study population consisted of pregnant women in first trimester in two different Norwegian counties (Sør-Trøndelag and Buskerud) and the period of inclusion was from August 2010 to May 2011 (until 1000 samples at each site). The cross-sectional design is not ideal in examining associations between exposure and the outcome (155). However, in our study, we only aimed to find the frequency of toxoplasma IgG positivity in our population. Therefore, the cross-sectional design was suitable.
The size of the study sample and precautions taken in our laboratory, being discussed later, are additional strengths of the study.

Limitations

First, prevalence is a "snap shot" of the presence of one particular outcome at one defined time point (155). Our study tells us the toxoplasma IgG prevalence from August 2010 to May 2011, a period prevalence. In terms of toxoplasma immunity, the prevalence is an estimate of accumulated exposure during a person's life so far, because the immunity (IgG positivity) persists. We, therefore, expect the prevalence to be representative for a longer time span. The prevalence has been relatively stable for the last 40 years with a stable incidence of maternal primary infection between the early1970-ies and early1990-ies (24, 49). We do not know the current incidence.
Second, repeated cross-sectional studies raise challenges, because the methods and population may change over time (155). Our study and the previous Norwegian studies have not included pregnant women from exactly the same areas (Figure 9) (23, 49, 156). Mid-Norway, Møre-Romsdal and Sør-Trøndelag are comparable areas, but differences between the estimated prevalence may be due to geographical differences. The present study and the study of Jenum et al. used the same laboratory method (23); however, the cut-off values used were slightly different because the manufacturers change of reference values. In our study, very few sera had values close to the cut-off values. Thus, if the prevalence was calculated with cut-off value of 6 IU/ml as in the study by Jenum et al., the prevalence was not changed. In the study by Stray-Pedersen and Lorentzen-Styr the dye test was used and small differences in the estimated prevalence's may also be caused by different diagnostic methods (49). Prevalence rates may vary, according to the sensitivity of the selected diagnostic method, as noted in studies comparing diagnostic assays (157).

Third, we had little information about the women. Information on ethnicity, parity and education would have provided us with useful information judging possible selection bias, i.e. whether our cases were representative of the source population. Because of the sample size and consecutive enrolment, we have minimized this possibility. See the discussion below.

Reliability/precision

Reliability refers to what extent repeated measurements will give the same result under identical conditions (155). "*Lack of reliability may arise from divergences between observers or measuring instruments or instability of the attribute being measured*" (1).

Precision is an alternative expression that describes the extent to which repeated measurements will give the same result. The precision gives an estimate of the variation around a defined value of a test and describes the closeness of agreement between multiple test results (155, 158). The CV % of laboratory analyses, i.e. the EIA-test, indicates the precision of the method.

<u>Random errors</u>

"errors due to the play of chance leading to lack of precision"(1).
Random errors or the proportion of variation in a measurement that has no apparent connection to any other measurements or variable, can reduce the reliability of the data or the degree to which the results can be repeated (1). Good precision in measurements and adequate sample size are the main tools to reduce random error (154). Increasing the sample size of a study population reduces the variability and thereby, increases precision (155).

In our study, the power estimate was performed before the start of the study and was based on the prevalence estimated 20 years ago (10.9%) (23). The sample size was sufficient to answer the main aim. However, when estimating the prevalence in the six age-categories, particularly for the oldest and the youngest, the number of included women was too small to obtain robust estimates.

Validity

Validity in research refers to whether we measure what we intended to measure and whether the results obtained answer the questions asked (155, 159).

<u>Internal validity</u>

"the degree to which a study is free from bias or systematic errors"(1).
The source of systematic errors may come from selection bias, information bias or

confounding factors (154). Bias undermines the internal validity of research (159). Internal validity is a precondition for external validity (154).

Selection bias

"error due to systematic difference in characteristics between those who take part in a study and those who do not" (1). Selection bias may also exist between groups within a study, for example, if the cases and controls differ importantly aside the disease or main outcome in question (159). Presence of selection bias may result in difficulties concerning comparability between the groups being studied.

Study population

As snap shots, the prevalence tells us about the background population; however "*the image may be blurred"* if the selection criteria of the study population are not appropriate (36). The chosen study population is important when estimating the prevalence; is it representative of the pregnant population in the selected counties? The women were not randomly selected, but the serology samples were sent from every general practitioner and midwife in the region taking care of pregnant women during the stipulated time period. In the laboratories the sera were collected and sent to the hospital laboratories consecutively. This implied inclusion of all women having the first antenatal control in Buskerud or Sør-Trøndelag during this period, except from the women with lost test tubes or test tubes containing too little serum for the ELISA analysis. In total, 78 women were excluded; 71 from Sør-Trøndelag and 7 from Buskerud. In one woman, year of birth was missing, 36 test tubes were missing for unknown reasons and 41 tubes contained too little serum for the toxoplasma IgG test. We have no reason to believe that the missing test tubes represent a certain group of pregnant women and believe that the pregnant women in our study are representative of the two regions. The proportion of missing results of about 4%, does not threaten the validity of the study. Furthermore, we do not expect systematic differences of the sampling in the two counties. Low number of participants is an important source of selection bias (155). In our study, the serologic samples were collected consecutively, until the estimated needed number of 2000 pregnant women. Therefore, we do not consider this as an issue in this study.

Seasonal enrolment

One limitation of our study may be the time of the inclusion. The women in the study population had their estimated date of delivery within the period March – November, 2011. In Norway, many women plan their delivery date to fit admission to kindergarten, which accept

one year old child born before September the previous year. This may be one of the causes to the birth boom during the summer (http://statistikk.fhi.no/mfr) , (http://www.aftenposten.no/norge/Fire-mulige-arsaker-til-at-det-fodes-flest-barn-om-sommeren-33023b.html) (160). Possibly women with higher education, who plan to go back to work after maternity leave, are over-represented in our study sample and that the sample includes less immigrants. This could influence the estimated prevalence. Equivalent studies as our have reported an increased prevalence among women with higher education; hence, the prevalence might be falsely high (161). On the contrary, several studies have found a higher prevalence of toxoplasma IgG among immigrant populations (23, 50, 162). Due to the long inclusion period and the large sample size, these possibilities, even if true, would make no great impact on the prevalence rate.

Another issue concerning the time of enrolment, is the possibility of seasonal changes in toxoplasma IgG prevalence. Seasonal changes in incidence of toxoplasma infection have been reported in some studies (163, 164). One of the strongest risk factors for getting *T. gondii* infection in pregnancy in Norway, as reported by Kapperud et al., was eating undercooked mutton (37). The autumn is the season for lamb slaughtering in Norway, leading to a higher rate of consumption of fresh and often pink lamb meat. However, we do not expect this to have great impact on our prevalence findings, because prevalence is a result of accumulation over time. The lamb season is short and most pregnant women take precautions. The study sample is large and the inclusion continued throughout winter and spring.

Information bias

"has information been gathered the same way?"(159).

Information bias occurs when the variables of interest, that is, the main exposure, covariates and the outcome, are measured with measurement error or are misclassified (154). This may occur both during collection of data as well as during coding and processing of data. In order to minimize information bias, information on outcomes should be gathered in the same way for all study participants (159). This is a type of systematic errors, which may influence the results, i.e., if the laboratory equipment is used incorrectly, not calibrated properly or any liquid used in the test, (for example the ELISA-test) is old, damaged etc. Measurement bias can be reduced by good quality control and training of staff. To avoid this in our study, all samples were analysed by two skilled technicians, using one analysing instrument in one laboratory, at the Department of Laboratory Medicine, Section of Medical Microbiology at Vestre Viken Hospital Trust. Furthermore, the results of the toxoplasma ELISA IgG analyses

were validated against the independent controls included in each run. Additionally some samples were analysed in the next run to check the level of agreement between the test results.

Clinical validity

”the ability of a test to correctly identify a person who does or does not have the disease of interest” (1). This term encompasses both sensitivity and specificity.

The analysing method used in a prevalence study needs to be accurate, including only the true positive individuals in the estimate. However, almost all tests fail to provide perfect separation between those with and those without disease (154). The EIA-assay used in the study has been evaluated in several studies and is widely used (95, 165, 166). Hofgärtner et al. observed a sensitivity of 95.6 % and a specificity of 98.7 % in the Platelia Toxo IgG test in their study, comparing four different assays to detect toxoplasma IgG in serum samples (166) . A combination of different assays may increase the sensitivity or the specificity (154). In a clinical setting of suspected toxoplasma infection, the Platelia Toxo IgG test result (or other assays) will be confirmed by different assays in order to increase the sensitivity or specificity and to estimate the time of the infection as accurate as possible.

External validity:

“to what degree the findings of the study can be generalized to groups that did not participate in the study” (1).

The prevalence of toxoplasma IgG positivity among pregnant women varies between different geographical parts of the world, and differs even among inhabitants living in one city (36, 162). Therefore, generalisation of toxoplasma IgG prevalence’s should be done with caution. The reasons for this variation are many and include climatic and hygiene measurements to animal keeping and diet and to the laboratory methods and cut-off values used (36). Our estimated prevalence can therefore only be applied to Norwegian counties, similar to Buskerud and Sør-Trøndelag. However, as seen in Table 4, several demographic variables in Buskerud and Sør-Trøndelag are representative of the total Norwegian population. Nevertheless, in the study of Jenum et al. 1992, they found a much higher IgG prevalence in Southwest Norway (13.4%) compared to Northern Norway (6.7%), explained both by climatic differences with wet moist weather in southwest, compared to the artic climate in the north and to the international population in one of the largest cities in Southwest Norway (23).

Table 4. Demographical data from the total Norwegian population and from Sør-Trøndelag and Buskerud counties in 2011

Variable	Norway	Sør-Trøndelag	Buskerud
Population			
Population number, n	4 920 305	294 066	261 110
Population growth per year, % (range)	1.33 (0.43, 2.35)	1.31	1.55
Female population, n (%)	2 431 447 (49.4)	146 097 (49.7)	130 922 (50.1)
Immigrants, % (range)	13.1 (7.2, 30.4)	9.2	14.7
Life expectancy; women, years (range) [#]	83.1 (77.8, 83.5)	83.1	82.9
Median population age, years [¤]	39.4	42.2	40.0
Infant death < 1year, per 1000 born	2.3	2.7	2.1
Fertility rate[¥]	1.82	1.86	1.82
Birth weight: <1500g, %, range	0.9 (0.5, 1.4)	0.9	1.1
Birth weight: >4500g, %, range	3.2 (2.3, 4.6)	3.1	2.3
Living conditions			
Average income, nkr (range)	376 300 (338 700, 426 600)	370 500	360 000
Low income households, % (range)	9.6 (7.2, 14.8)	9.1	9.7
Unemployment, 15-29 years, %	2.9	3.1	2.9
Unemployment, 30-74 years, %	1.9	1.9	1.9
Disabled (%)	3.9 (2.6, 5.6)	4.1	4.4
Higher education (≥9 years) (%)	83.1 (76.3, 86.5)	86.5	80.4
Living habits			
Travel abroad (%) (SSB)	48.2 (43.4, 51.7)	52.7*	58.8**
Eating fruit and vegetables daily (%)	50 (41, 55)	51	55
Physical activity, ≤ 1 hour per week (%)	26 (19, 33)	26	22

SSB= Statistics Norway [Statistisk Sentralbyrå]. Numbers from http://www.norgeshelsa.no/norgeshelsa from 2011 or https://www.ssb.no/befolkning from 2011 unless other stated, # 2012, ¤ 2014, ¥ mean number of births per women during life, * Trøndelag (Nord-Trøndelag + Sør-Trøndelag), ** Oslo and Akershus county.

A higher prevalence of toxoplasma infection is described among non-western immigrants in several studies (23, 36, 50); therefore, a large change in immigration from non-Scandinavian countries may change the prevalence of IgG rapidly.

7.1.2 Papers II and III

In descriptive studies, the purpose is often to explore the characteristics of a group of subjects. There is no testing of casual hypothesis or comparisons with other groups, but the studies may expand the understanding and document facts of clinical or theoretical interest (http://www.oxfordjournals.org/our_journals/tropej/online/ce_ch4.pdf).

Strengths and limitations

Strengths

A cohort study has an observational design in which a *"defined group of individuals are followed or traced over a period of time"* (154). A cohort study may be prospective or may be retrospective, the latter is sometimes referred to as historical cohort studies (154). In the typical retrospective or historical cohort study, the health records have already been collected and stored and the cohorts are identified by recorded information (167). The risk of disease occurs before the start of the study and the advantage is that the exposure is recorded before the occurrence of the disease. The second part of our project can be considered as a retrospective cohort study in which pregnant women are followed from time of first toxoplasma serology until delivery of their offspring. The advantage of these studies is that the data are already available, as in our study (153).
Furthermore, descriptive studies give the possibility to study rear outcomes. Antenatal toxoplasma infection in Norway is infrequent. We do not know the current incidence of maternal primary toxoplasma infection, but based on findings from early nineties relatively few maternal and foetal infections occur per year (24). In order to gain knowledge on this small patient group, we explored patient lists and laboratory records to find all pregnant women with suspected toxoplasma infection undergoing amniocentesis at Oslo University Hospital. This enabled us to include a substantially large sample of women, 346 singleton pregnancies in the second part of our study (paper III) and 176 women in the avidity-study (paper II).

Other strengths are the laboratory precautions that will be discussed later, follow-up throughout pregnancy of most women and thorough recording of laboratory test results in all the women.

Limitations

A clear, specific and measurable case definition is important in descriptive epidemiology (153). However, some diagnoses are challenging and depend on a cluster of factors rather than one stringent definition. The diagnostic criteria's of maternal and foetal toxoplasma infection is defined by Lebech et al. (90). In some circumstances, the diagnosis is fairly easy, for example when maternal seroconversion or positive toxoplasma PCR occurs. Often, the time of infection and occurrence of foetal infection is more ambiguous and several factors are used to conclude, mainly kinetics of different specific tests during follow-up of pregnancy. To

overcome this challenge, two experienced and dedicated experts on toxoplasma infection in pregnancy analysed all test results in order to ensure diagnosis and estimate time of infection. A consequence of using existing data is that all important information may not have been obtained (154). The data was recorded by different health care professionals being involved in patient care throughout the twenty-one years inclusion period. This may have resulted in a less accurate database than that achieved in a prospective cohort study (167). In a retrospective cohort study on lifestyle factors and cardiac disease, Fouwels et al. found that even in patients at risk relevant information on lifestyle habits was lacking or incompletely registered in patient records (168). In our study, information on type and duration of antiparasitic treatment, symptoms of and risk factors for obtaining toxoplasma infection were occasionally lacking. However, we obtained complete data on laboratory results during pregnancy and birth as well as information on maternal age, nationality and GA at serological testing, amniocentesis and at birth.

Retrospective or prospective cohort studies often have long inclusion periods. Our patient group was collected over a period of 21 years, which leads to heterogeneity within the study population. The diagnostic techniques evolved during the study period, in particular with the introduction of IgG avidity, possibly resulting in a decrease in the number of amniocenteses over time. In our study, the management of women and children did not change substantially during the period other than a reduced rate of amniocenteses and a lower rate of toxoplasma testing during the first year of life.

A prospective design would make it possible for us to control information gathered from the women better than in our study, but we would need inclusion for several years or a large study sample to obtain a sufficient number of women. This would be time-consuming and expensive.

The case-control design could be used to answer certain aims, that is, complication rate after amniocentesis. This was initially planned as part of the project. However, complications except miscarriage were seldom noted in the patient records which made the occurrence of procedure related complications very low. We therefore did not complete this intended part of the study. The finding of few miscarriages is reliable, but because of the low number of cases and lack of a control group we have no power to conclude that the foetal loss rate is lower than in studies, in which a larger volume of amniotic fluid is obtained. Robust findings would need a larger study sample and a control group.

Reliability/precision

<u>Random errors</u>

The database contained 130 variables and errors might occur in typing or registration, even if the data base was checked (data cleaning) thoroughly by an IT-advisor before the data analyses.

Twenty-one laboratory methods were in use during the 20-years period (Table 5, Appendix). All laboratory methods have a possibility of inaccuracy. However, women with suspected toxoplasma infection were tested with a panel of different tests and a follow-up sample was also tested with the same assays at the reference laboratory in order to assure the diagnosis.

In the avidity study (paper II), inaccuracy and differences in laboratory measurements are seen, as the avidity-value fluctuated in several women (paper II, Figure 1). This may be explained by inter-assay variation because the patients' sera were tested consecutively during pregnancy and not tested simultaneously. We defined a significant increase in IgG avidity ratio to be >5% or >0.1 AI.

Validity

<u>Internal validity</u>

Selection bias

Our samples included the total number of singleton pregnancies undergoing amniocentesis because of suspected toxoplasma infection at Oslo University Hospital (346). Selection bias is most often not relevant in retrospective cohort studies because the subjects are already present in the records (http://www.oxfordjournals.org/our_journals/tropej/online/ce_ch4.pdf), however, we cannot exclude the possibility of selection prior to referral to our centre. In our study, there may be a selection in the patients being tested and referred either because of own request or because they are risk patients (see discussion in section 7.2.3).

Information bias

Variables like symptoms of and risk factors for toxoplasma infection, such as eating raw meat, traveling to countries outside Scandinavia or contact with cats, cannot be explored in our study. The doctor may have asked the women with suspected toxoplasma infection more thoroughly, and the women themselves may remember or focus more selectively on known symptoms or risk factors. This may be regarded as recall bias which we had to take into consideration if we were to compare our study population with other pregnant women. In addition, while we lack a comparison group, we do not know how often traveling to countries

outside Scandinavia occurs among pregnant women in Norway. Studies without a comparison group cannot be used to conclude on the risk and cause of a disease (154).
The questionnaire sent to women with unknown GA at birth (Appendix), was not validated. The form contained information about birth mode, GA, neonatal weight and length, in addition to questions on neonatal antiparasitic treatment, health at birth and first year of life and current health. When using an invalidated questionnaire, the women might have misinterpreted the questions. In the end, we only used GA at birth, neonatal weight and sex. These parameters are accurately remembered by women, even after many years (169) .

Clinical validity

The interpretation of the IgG avidity results in paper II needs to be related to the type of assay used and the chosen cut-off value, as no gold standard exists. Discrepant results between different assays are reported by several researchers (170-172). This implies that when interpreting IgG avidity results and other laboratory results, the type of assay, the sensitivity and specificity have to be taken into consideration. Our decision on cut-off values for significant changes in avidity was dependent on the calculated CV% of the kit controls which was <10% for the IgG index value.

External validity

Our study is a descriptive study on a restricted population; women undergoing amniocentesis due to suspected toxoplasma infection at Oslo University Hospital. Our findings cannot be generalized to all pregnant women in the Southeast Health region. However, mean age at birth is fairly similar to the general birth age in our region and the proportion of non-Scandinavians in our study is lower than expected (173, 174). The educational level seems higher (69.2% with education >12 years); however, concerning this variable, we lack information on several women. Our aim was to describe our cohort in order to assess our procedures and follow-up, and even if the study population is not representative of the background population, our findings concerning testing and follow-up is clinically relevant in a broader setting than ours. However, the findings can mainly be applied in other Scandinavian countries or in countries with similar toxoplasma IgG prevalence among pregnant women.

Statistical issues

Dichotomizing continuous variables lead to loss of information and reduces power (175). In paper I, age are coded as a continuous variable, but was further polytomized in to six five-year groups, similar to the once in the study by Jenum et al. from 1993-1994 in order to ease comparison (23).

Small sample size reduces the ability of detecting small effects as in the youngest and oldest age category in paper I, and thereby causing type II mistakes (176). The small sample size leads to loss of precision in these groups.

Borderline antibody result (IgG 4-<8 IU/ml) was detected in 18 women in the prevalence study. Borderline value means values in the grey zone, where the values may be categorized as positive or negative. We decided to include them in the IgG negative group in the estimation of prevalence, in order to avoid overestimation of the prevalence. If the borderline results are included in the equation as IgG positive, the prevalence will not change substantially (10.2%). The most correct approach would perhaps be to exclude them from the study or calculate the percentage of all three groups (IgG negative, borderline, IgG positive). This would not give a substantial change in IgG prevalence (179/1904=9.4%).

7.2 Interpretations of the main results

7.2.1 Paper I

The prevalence of toxoplasma IgG among pregnant women in Norway is still low (9.3%). Consequently, 90.7% of the women are uninfected and susceptible to primary toxoplasma infection in pregnancy. Our estimated prevalence does not necessarily reflect the prevalence among pregnant women in all regions of Norway because of geographical differences and because our study does not represent a random selection of pregnant women in Norway. However, we expect the results obtained from each county to be valid (as discussed previously). We also expect the national prevalence of toxoplasma IgG among pregnant women in Norway to be close to the estimated prevalence in Buskerud and Sør-Trøndelag (Figure 9).

Despite the challenges in the interpretation of results from serial cross-sectional studies, the prevalence in Norway seems to have been fairly stable over the last 40 years (Figure 9). This contrasts to findings in other European studies where a marked decline in toxoplasma IgG seroprevalence is seen during the last decades (39-41). However, the Norwegian prevalence was low already during the seventies and has been lower than in most Scandinavian countries (49). To the best of our knowledge, there is no updated prevalence of toxoplasma IgG in pregnancy from the other Nordic countries for comparison.
It may be that factors pointing towards reduction in prevalence, such as increased awareness by the pregnant women and the caregivers, better animal hold and hygiene are neutralized by larger proportion of non-western immigrants and increased risk behaviour in the Norwegian

pregnant population (e.g. traveling to countries outside Scandinavia and French diet).
A Norwegian study from 2003 reported a prevalence of toxoplasma IgG among military recruits to be 8.1 %. The study included 620 recruits of whom 4.3% were women, at age 19.6 ± 1.6 years (177). The results confirm a low prevalence among young Norwegians. The study should be interpreted with caution since the study mainly consists of males and the included number is small for estimating prevalence. In addition, the study does not give the geographical location and recruitment method.

There are recent concerns in infectious medicine on the influence of climatic changes on pathogens (178-181). Climatic changes may affect the toxoplasma prevalence. Both higher temperature and high humidity may increase the survival of toxoplasma oocysts and thereby increase the risk of infecting animals and humans (182). Three main weather trends are postulated for the northern Europe; i. Increase in total precipitation (wet weather type), ii. Increase in total temperature, iii. Increase in weather extremes (183). In addition, weather changes may cause alterations in animal ecology like eating habits and animal migration which for example, may cause transport of the parasite to more uncommon areas. The last decade *T. gondii* is found in artic foxes, walruses, birds and the polar bear (184). Therefore, continuous surveillance of the toxoplasma prevalence in humans is of interest.

The prevalence was fairly stable across the largest age groups (Table 2), indicating that most of the women were infected prior to pregnancy and that few pregnant women acquire infection during pregnancy. The measured prevalence of approximately 10% among pregnant women about 20 years of age, means that on average the prevalence in this group has increased by 0.5% yearly since birth. The fact that the prevalence does not increase significantly thereafter, means that the risk of being infected may have decreased in the susceptible group of pregnant women. This is supported by previous findings of low incidence during pregnancy (24). We found that the median IgG antibody level was highest in the very young age group (≤19 years) and lowest in the oldest age group. Since the antibody level normally will decline with time after infection, this might indicate that infection occurs earlier in life. The prevalence of IgG antibody positivity was highest in the group of women 40 years or older (Table 2). The higher prevalence in the oldest group may rather represent a cohort effect than a specific life style among women ≥ 40 years with increased risk of infection (e.g. playing with kids in the sand, ability to travel and eat in restaurants). Jenum et al. in 1992-1993 obtained the same results, though from the age of 35 years (23). However, the incidence of primary toxoplasma infection in their study was not higher in the oldest

group compared to the total group which indicates a cohort effect (24). The lack of an increase in incidence with age in the study by Jenum et al. may additionally be explained by the lower number of women in the oldest age group. This may indicate that most of the infected women acquired the infection earlier in life, possibly in their teenage years in which travel and eating habits may change, and that the frequency of new infections is fairly stable thereafter. Nevertheless, as previously stated, the number of women in the youngest and highest age groups in our study are low and our findings must be interpreted with caution due to lack of power, as seen by the wide confidence intervals in the IgG-values in the youngest and oldest age groups. The finding may only be regarded as a tendency confirmed by a previous study (23).

7.2.2 Paper II

Of the 352 women included in the study, 176 had repeated toxoplasma IgG avidity measurements for over three months or longer during pregnancy, most of them followed from GW 11 to childbirth. IgG seroconversion was seen in 17 women and 31 had IgG antibody increase, both indicative of primary toxoplasma infection (90). However, 72.7% (n=128) had positive IgM and low IgG avidity. IgG avidity development is of utmost interest in this latter group.

The finding that the IgG avidity remained below the high avidity threshold level in 79% (139/176) of the women, at a median of seven months, confirms previous studies describing long duration of low IgG avidity in pregnant women (88, 89, 185, 186). In 42% of the women, the IgG avidity remained low and stable during pregnancy and in 35.2%, the avidity increased significantly but was still below the high threshold level (Figure 12).

The time of infection in most of the women was unknown, because they already had low IgG avidity in the first serology, neither did we follow all until high avidity developed, because the last avidity test was collected at the end of pregnancy. Nevertheless, researchers have estimated the duration of low avidity from four months in pregnant women up to 14 months in women included in pregnancy and followed into maternity with known onset of infection (89, 172, 185). The variation in duration of low avidity in the different studies may be caused by several factors; individual pattern of immunoglobulin kinetics modulated by factors such as pregnancy or treatment, or by different assays and cut-off values used (170, 172, 187-191). In a study comparing 32 pregnant women with seroconversion and antiparasitic treatment and 16 non-pregnant untreated adults, a significant delayed maturation of the IgG avidity was

reported in the pregnant group (188). Increasing avidity after delivery and discontinuation of treatment was described. It seems not evident, however, whether the slow development of avidity was caused by pregnancy or treatment. In our study, most patients received a macrolide (spiramycin or azithromycin) with median treatment duration of 21 days, meaning that most of their pregnancy proceeded without antiparasitic treatment. Despite this, maturation of IgG avidity was slow in most women. There was no significant difference between the women with increasing or stable avidity concerning the use and duration of antiparasitic treatment. Most of them received treatment and the sample size is too low to conclude.

As shown in paper II, Figure 1, there is a great diversity in the development of the IgG avidity throughout pregnancy. The development is not strictly linear. In studies by Meroni et al. and Jenum et al., similar curves are reported (89, 188). The nonlinear pattern of IgG avidity development seen in several patients may be due to inter-assay variation, because the sera were not tested simultaneously. Individual immunological differences may be present as well.

The finding of long duration of low IgG avidity in our group of women, even with unknown time of infection, highlights the core problem in clinical daily life. Timing the onset of infection is crucial because acquisition during pregnancy poses a threat to the foetus.

High maternal IgG avidity in first trimester is a strong indicator of the infection being acquired before conception (192). The ideal situation for assessing time of infection would be a toxoplasma serology, sampled just before conception or very early in pregnancy. Usually this is not possible, because we do not have a national toxoplasma screening programme and because the first antenatal check-up according to Norwegian guidelines should take place between 8 and 12 GW (http://legeforeningen.no/Fagmed/Norsk-gynekologisk-forening/Veiledere/Veileder-i-fodselshjelp-2014). Therefore, when pregnant women have their first toxoplasma serology in GW 11, with unknown toxoplasma status prior to pregnancy and low IgG avidity, it may be a challenge to assess the need for antiparasitic treatment, amniocentesis and further follow-up.

There were four infected offspring in this sub study, however, none among the women with IgM positivity and stable low IgG avidity values, without seroconversion or titre increase. In these cases the likelihood of preconceptional infection is large. This finding, however, must be interpreted with caution because of the low number of infected offspring (n=4). To the best

of our knowledge, there are no previous reports on foetal outcome in relation to IgG avidity development over time in the mother.

IgM and IgG antibodies and IgG avidity are still our best tools in assessing the time of toxoplasma infection. However, we must be careful not to unnecessary start antiparasitic treatment, additional examinations and follow-up throughout pregnancy and first year of life. Therefore, due to difficulties and the importance in assessing time of infection in pregnant women, we advocate looking at avidity maturation in addition to IgG and IgM changes. One single serum sample must be confirmed and compared with a new sample after three weeks, and possibly an additional test should be performed three weeks later in cases with inconclusive serology before offering treatment, amniocentesis and further follow-up.

7.2.3 Paper III

Our main aim was to evaluate the use of amniocentesis in our population with low prevalence of toxoplasma IgG and with no antenatal serological screening program. We wanted to examine whether amniocenteses were performed on the correct patients and whether amniocentesis is a safe procedure on this indication. In terms of "correct" patients, three issues are relevant; firstly, are we misdiagnosing referred patients, thereby, performing unnecessary amniocenteses? Secondly; do we fail in performing amniocentesis in patients with infection due to misinterpretation of the serologic analyses? Thirdly, are there risk groups where toxoplasma serology has not been performed? Possibly all scenarios occur. The first issue can be addressed by our study. For the second and third issue, we can only speculate based on epidemiology and previous studies. In the following section, I will discuss the first issue, thereafter, the second and third and at the end, elaborate on the infected foetuses.

Firstly; in 27% of the women infection was acquired during pregnancy, thus, these women were correctly referred and underwent further treatment and follow-up according to our guidelines. However, we observed that maternal infection most likely occurred prior to conception in 50% of the women. As earlier stated, the categorization of women according to time of infection and serologic group was performed by two dedicated experts individually, according to Lebech's criteria (90). This involves a certain degree of inaccuracy, yet consensus was reached for interpretation of the results. Even though errors in the evaluation may have occurred, we still believe that close to half of the women acquired the infection before pregnancy and therefore, underwent an unnecessary amniocentesis. This finding is

supported by the study of Prusa et al., where 45.4% of the women were considered to be infected prior to pregnancy and were excluded from further analyses (193).

One reason for performing amniocentesis in women with latent infection is misinterpretation of the toxoplasma serology. The introduction of IgG avidity in the nineties may be the cause of reduction in unnecessary amniocenteses during the last part of that decade. However, contrary to the following years, the clinicians may have laid too much emphasis on the low avidity in predicting primary infection. To differentiate between latent and primary infection may be difficult because 23.1% of the women were categorized as "possibly infected" during pregnancy by our experts, despite the light of retrospectivity.

If antenatal toxoplasma infection is suspected, the woman is exposed to amniocentesis, antiparasitic treatment, follow-up and further testing during pregnancy, delivery and of the neonate during first year of life. As a clinician, it is important to be aware of the burden for the patient caused by this algorithm. Antiparasitic treatment may cause side-effects in the mother or her foetus and raises the question on loss of "microbiome" (endogenous microbes). Recent research has highlighted the importance of "the microbiota" on maternal and foetal health outcomes, including immune and metabolic function in the child later in life (194-196). The natural microbial ecosystem will not return completely to baseline after a course of antimicrobial treatment with possible effects on the maternal and fetoplacental microbiomes (194). Thus, there are good reasons to withhold antiparasitic treatment when not needed.

Monitoring possible foetal pathology throughout pregnancy causes parental worries, particularly if the prognosis is ambiguous. This may have a negative impact on the foetus (197-200). One of the pregnancies in our study was terminated due to maternal emotional distress. The foetus had no detected structural abnormalities and the amniotic fluid toxoplasma PCR was negative. Berrebi et al. stated that the only indication for termination should be severe structural abnormalities in infected foetuses (201). However, women may decide to terminate their pregnancy, despite the absence of ultrasound findings, because of uncertainties and worries. Therefore, it is important to avoid unnecessary diagnosis and follow-up in women with latent infection.

Several researchers view the second trimester amniocentesis as relatively safe, with low degree of procedure-related complications (117, 119, 202). In our study, we detected two miscarriages. Both took place four weeks after the procedure that were performed in GW 13. One of these two foetuses was severely infected with an isolated large risk of intrauterine

death caused by the toxoplasma infection (5). It is therefore unlikely that this miscarriage was caused by the procedure. The other miscarriage *may* be related to the amniocentesis. Today, we do not perform amniocentesis in GW 13 due to the higher risk of procedure-related complications at this gestational age, compared to a few weeks later (117, 202). We obtain less amniotic fluid compared to amniocentesis performed on genetic indications. Few studies have examined the relationship between the foetal loss rate and the amount of amniotic fluid aspirated, but the results from two studies indicate a lower foetal loss rate if less fluid is obtained (203, 204). One study lacked a control group and the other study had non-significant findings. Therefore the impact of the amount of amniotic fluid removed on foetal loss after amniocentesis mainly remains unanswered. With our study design, we cannot conclude on the frequency of post amniocentesis complications. An investigation of foetal loss rate following amniocentesis performed due to toxoplasma infection with less fluid removed, would require a control group. A large number of pregnancies would be needed in order to have adequate power because the current foetal loss-rate is low.

Secondly; possibly we have underdiagnosed some women with primary infection which have been evaluated to have latent infection and therefore, have not undergone amniocentesis. Moreover, we may have categorized some children with lack of follow-up in the first year of life as uninfected because of negative PCR after amniocentesis.
Only 15 infected offspring were detected during the 21 years study period. This indicates 0-2 cases per year which are in line with the reported number of cases from several countries with a surveillance system, but without screening programmes (Annual epidemiological report. Food-and waterborne diseases and zoonosis 2014.www.ecdc.europa.eu). However, there are reasons to believe that the number is higher, because France which has both surveillance and screening systems reported 186 cases of congenital infection in 2011.

This leads us to the third issue; are there women who should be referred, but who are not? The prevalence of toxoplasma IgG among pregnant women has been relatively stable during the last forty years (see previous discussion) (23, 49, 156).The current incidence of maternal toxoplasma infection in Norway is unknown. It seems to have been stable between the seventies and nineties and we found few women with high toxoplasma IgG antibody-levels in our study in the first part of our project. We therefore expect the incidence of maternal infection to still be low. The incidence of congenital infection will be even lower. However, despite low incidence of maternal and foetal infections, we would expect more infected cases in our health region covering approximately 50% of the Norwegian fertile female population

(205). Through the yearly reports from the Toxoplasma Reference Laboratory (http://ous.prod.fpl.nhn.no/fagfolk_/laboratorietjenester_/nasjonale-referansefunksjoner_/Sider/Nasjonalt-referanselaboratorium-for-toxoplasmose.aspx), there is information on at least ten additional cases with congenital toxoplasmosis in the Southeast Health Region during the last decades. However, these cases were not included in our study since amniocentesis was not performed; eight were detected late in pregnancy and two were detected because of intrauterine foetal death. This indicates that toxoplasma infection exists and that there are cases not being detected or being detected too late for treatment and follow-up. The reasons for not detecting these cases may be several. One obvious reason is that we do not have a toxoplasma screening system in pregnancy in Norway. Eight of the infected offspring in our study and six of those infected without amniocentesis being performed were detected during 1993-1994 and the subsequent years, that is, during and following the toxoplasma screening project in Norway from 1992 to 1994 (23). Like most other European and Western countries, we have a risk based approach, testing women at risk of infection or with typical symptoms as enlarged lymph nodes. However, most of the toxoplasma serology analyses are performed on women's request or due to the assessment of the health care provider at the first antenatal control. The number of samples sent to the Toxoplasma Reference Laboratory due to a positive IgM result are increasing (http://ous.prod.fpl.nhn.no/fagfolk_/laboratorietjenester_/nasjonale-referansefunksjoner_/Sider/Nasjonalt-referanselaboratorium-for-toxoplasmose.aspx), but the number of amniocenteses and infected offspring decreases. This may be explained by the fact that the samples are mainly collected very early in pregnancy and show a high IgG avidity on testing at the reference laboratory.

The proportion of non-Scandinavians in our material is lower than expected for the region, because the three large counties, Oslo, Akershus and Buskerud have a high proportion of immigrants (www.ssb.no/innvbef). The prevalence of toxoplasma IgG among immigrants is higher than in the native Norwegian population, a finding supported in other North European countries (23, 43, 50, 162). Additionally, there might be women refusing to undergo diagnostic amniocentesis and therefore not included in the present study. Possibly there are several immigrant women in this group (206, 207).

Some studies regard only persisting IgG after birth as confirmative of congenital infection (113). We considered positive PCR in amniotic fluid as a diagnostic sign of congenital toxoplasma infection because of the very low occurrence of false positive PCR tests. In the

studies by Hohlfeld et al. and by Wallon et al., the positive predictive value of the PCR test was estimated to be 100% and in both studies no false positive PCR results were detected (105, 107). In the study of Jenum et al., the specificity was 94.0% (102, 105). We did not have serology of all infected infants during the first year of life. Lappalainen reported three infants with retinal scars with no obvious postnatal positive serology. Therefore, to avoid the small possibility of a false positive or false negative result in PCR and serology, toxoplasma serology at birth, and during the first year of life, are recommended before categorization of offspring as infected or non-infected.

A range of ultrasound findings and clinical signs were detected among the infected offspring (10/15, 67%) (Table 3, paper III); most commonly intracerebral calcifications and chorioretinitis which are typical signs of congenital toxoplasma infection (5, 113). The proportions of findings were higher than in most published studies (79). However, in an American study of 164 cases with congenital toxoplasma infection, being referred to the Toxoplasma Serology Laboratory in Palo Alto (California), the proportion of severe findings was 85% (208). The most obvious reason for the high degree of clinical findings is the lack of a toxoplasma screening programme. The infection may have been detected late in pregnancy, as in our study (seroconversion at median 27.7 GWs), reducing the possibility of antenatal antiparasitic treatment earlier in pregnancy. Additionally, a screening programme as the French, may lead to serious malformations being detected early in pregnancy which often lead to termination of the infected foetus in local hospitals (201). Therefore, the reason that some European countries rarely report serious sequelae may be early termination of pregnancies with infected foetuses.

Most of the infected offspring had mothers with seroconversion or IgG antibody increase (13/15, 87%). Among these pregnant women, infection is relatively easy to recognize. The remaining two infected foetuses had mothers in the group with IgM positivity and low IgG avidity (2/255, 0.8%). One of the two women had no IgG avidity measured (in 1993), but had a very high IgG antibody level during the first serology, had several cats, recently been in France and had palpable submandibular lymph node. The other had low IgG avidity that increased during pregnancy, and had in addition submandibular lymph nodes. In this group, the challenge is, to assess time of infection as earlier stated.

Our study was not designed to detect risk factors. Because we were dependent on recorded notes, in several patients, symptoms and risk behaviour were not stated. However, a

substantial proportion of the women who were asked (170 of 234) had been travelling abroad, which is a well-known risk factor (37). Nearly 93% (158/170) of these had travelled outside Norway in the last six months before the first toxoplasma test, of whom 85% travelled to countries outside Scandinavia with higher toxoplasma prevalence than Norway. This finding reminds us of the importance of continuous education on risk factors as part of regular antenatal care. Foulon found a reduction in maternal toxoplasma infection (seroconversion) by introducing information campaigns intended for pregnant women (53).

8 Conclusions

The prevalence of toxoplasma IgG antibodies among pregnant women in Norway is low and has been relatively stable for the past 40 years, despite an increase in risk factors. The lack of increase in toxoplasma IgG prevalence across the age groups, indicate infection before pregnancy.

Our study confirms the previous finding that toxoplasma IgG avidity may remain low for several months during pregnancy. Moreover, together with the possibility of a long lasting IgM positivity and a first toxoplasma serum sample obtained at the end of first trimester, the time of infection may be difficult to assess. As a consequence the clinicians may perform unnecessary amniocentesis in a large proportion of women as shown in paper III. However, without seroconversion, antibody increase or avidity increase during the follow-up period the risk of foetal infection seems low.

If antenatal toxoplasma infection is suspected by serology, amniocentesis after 15 GW seems safe and useful as a diagnostic procedure to diagnose congenital toxoplasma infection.

9 Clinical implications

The present studies bring the knowledge and awareness of the parasite *T. gondii* to clinicians and caregivers via papers, lectures, oral presentations and posters.

Hopefully our knowledge of the current prevalence and the persisting diagnostic challenges will serve as a basis for closer collaboration between microbiologists at the Reference Laboratory and specialists in foetal medicine. Local and national guidelines should be revised.

The absence of toxoplasma screening and lack of sera sampled early in pregnancy may cause a challenging clinical situation. The long duration of the toxoplasma IgG avidity and large number of unnecessary amniocenteses detected, underline the importance of an experienced staff being in charge of women with suspected toxoplasma infection. Appropriate interpretation of serologic tests is best achieved by dedicated staff with adequate clinical information (i.e. GA at testing, presence of clinical signs, travel and exposition to other risk factors) (209).

Clinicians have to be aware of the possibility of the IgG avidity being low during latent toxoplasma infection. If the woman has positive IgM and low IgG avidity in the first trimester, a new sample should be obtained three weeks later for confirmation and assessment of

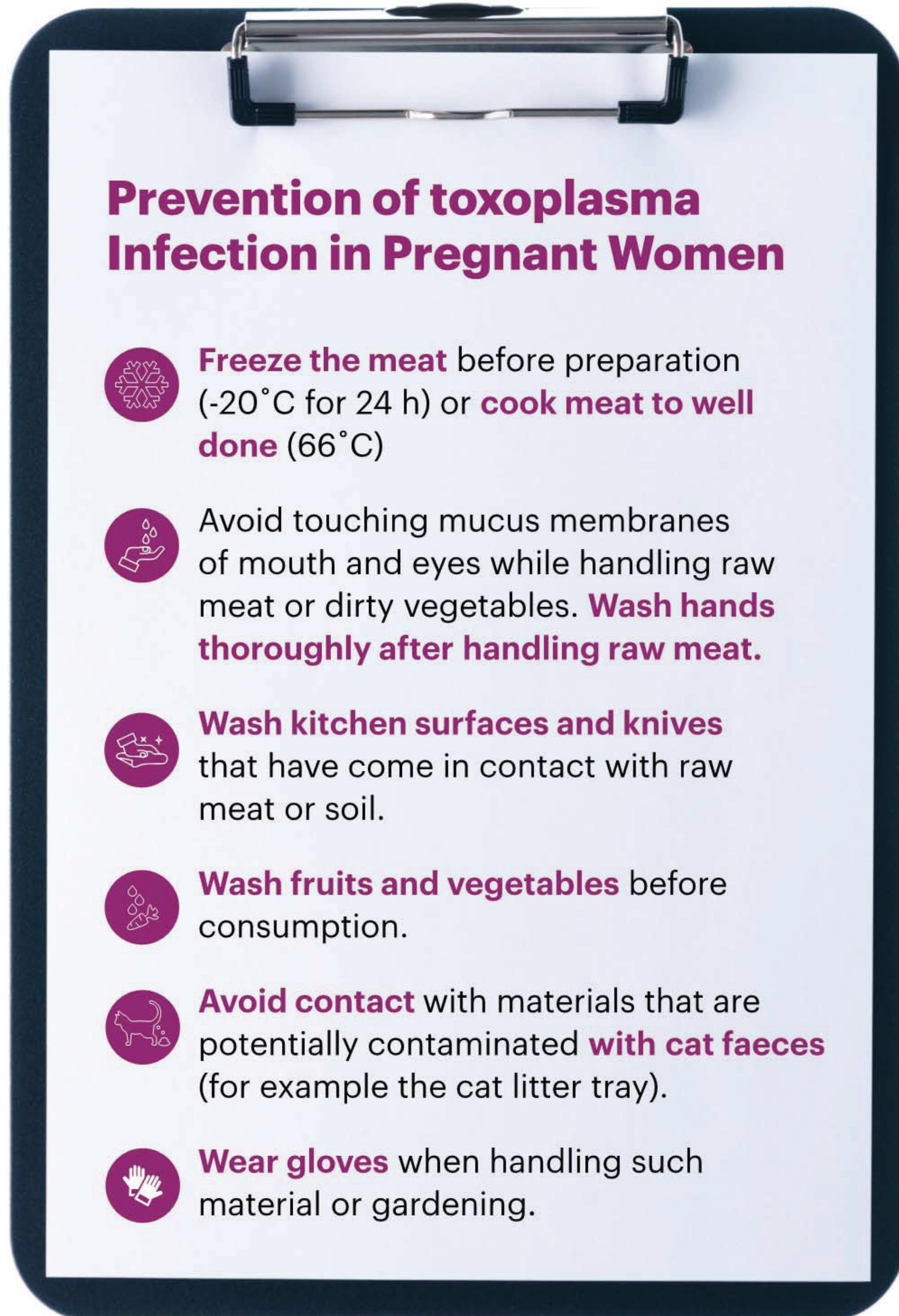
possible changes in antibody levels. In some patients, the final decision on amniocentesis can be improved by obtaining an additional sample after another three weeks to look for changes in avidity. It is important to note, however, that this advice mainly apply to women with serologic findings in first and early second trimester.

In risk groups, it is important to obtain a serum sample for toxoplasma serology as early as possible. For some patients, it is possible to analyse a preconceptional serum sample or a sample from early pregnancy since some laboratories store samples over a certain time.

In 82% of the offspring we found information on toxoplasma serology and PCR at birth and in 42% of the children, sera was collected during their first year of life. This number has decreased during the last decade. Because of the possibility, though small, of negative toxoplasma PCR in amniotic fluid, it is necessary to inform and remind health care givers and patients of the importance of serological follow-up after birth.

Congenital toxoplasma infection is a preventable disease. Since we lack a systematic screening programme, we are left with primary intervention as the principal means of reducing maternal infection and thereby lowering the number of foetal infections. Around 90% of the pregnant population in Norway is susceptible to toxoplasma infection. We therefore recommend thorough and continuing information to the pregnant population on how to avoid the risk factors for toxoplasma infection (Figure 11) (37, 38, 210). Studies suggest that education may contribute to a lower infection burden even if a substantial proportion of the infections may not be associated with recognized risk factors and might therefore seem difficult to prevent (53, 60). Because the first antenatal control in pregnancy usually takes place around GW 10-12, infection in first trimester is not prevented by information at the first antenatal check-up. Therefore the information should ideally reach the women before they become pregnant, for example by national educational campaigns. Targeted information during the antenatal controls should also be given.

Figure 11. Measures for prevention of toxoplasma infection in pregnancy (5)



10 Future perspectives

- The difficulties in assessing time of toxoplasma infection (papers II and III), reflect the potentials for further research on more specific tests. Research is still needed to improve the accuracy of diagnostic strategies.
- The Department of Medical Microbiology and the Department of Fetal Medicine have been working on toxoplasma infection for more than twenty years and we therefore consider our results to be relevant for clinicians. However, the knowledge on antenatal toxoplasma infection and other perinatal infections vary among clinicians. A knowledge center on toxoplasma and other perinatal infections is therefore warranted.
- Our cross-sectional study should be supplemented with prevalence estimated from other areas in Norway to get a closer picture of the toxoplasma IgG prevalence among pregnant Norwegian women. Ideally, a study should include pregnant women living in the same 11 counties as covered by the study of Jenum et al. As a minimum the prevalence in areas climatically and demographically different from Buskerud and Trondheim; like Rogaland in South-Norway, and Troms in North-Norway and the Oslo-Akershus area should be studied.
- A follow-up study of the offspring from project part two would be useful in order to assess long term risks and complications as an evaluation of our diagnostic and therapeutic management.

11 References

1. Porta MS, International Epidemiological Association. A dictionary of epidemiology. 5th ed. Oxford ; New York: Oxford University Press; 2008. xxiv, 289 p.
2. The American Heritage dictionary of the English language. 5th ed. Boston: Houghton Mifflin Harcourt; 2011. xxvii, 2084 p.
3. Laveran. On the endoglobular hematozoons of "Padda oryzivora". C R Seances Soc Biol Fil. 1900;52:19-20.
4. Nicolle C, Manceaux LH. On a leishman body infection (or related organisms) of the gondi. 1908. Int J Parasitol. 2009;39(8):863-4.
5. Remington JS MR, Wilson CB, Desmonts G. Toxoplasmosis. In: Remington JS KJ, editor. Infectious diseases of the fetus and newborn infant. 7th ed. Philadelphia, PA: Saunders/Elsevier; 2011. p. 918-1041.
6. Hunter CA, Sibley LD. Modulation of innate immunity by Toxoplasma gondii virulence effectors. Nature reviews Microbiology. 2012;10(11):766-78.
7. Weiss LM, Dubey JP. Toxoplasmosis: A history of clinical observations. Int J Parasitol. 2009;39(8):895-901.
8. Wolf A, Cowen D, Paige B. Human toxoplasmosis: Occurrence in infants and encephalomyelitis verification by transmission to animals. Science. 1939;89(2306):226-7.
9. De Sanctis AG. Advances in pediatrics. In: Sabin AB, editor. Toxoplasmosis. Chicago, etc.: Year Book Medical Publishers; 1942. p. 1-56.
10. Eyles DE, Coleman N. The relative activity of the common sulfonamides against experimental toxoplasmosis in the mouse. Am J Trop Med Hyg. 1953;2(1):54-63.
11. Eyles DE, Coleman N. Tests of 2,4-diaminopyrimidines on toxoplasmosis. Public Health Rep. 1952;67(3):249-52.
12. Eyles DE, Coleman N. Synergistic effect of sulfadiazine and daraprim against experimental toxoplasmosis in the mouse. Antibiotics & chemotherapy (Northfield, Ill). 1953;3(5):483-90.
13. Garin JP, Eyles DE. [Spiramycin therapy of experimental toxoplasmosis in mice]. Presse Med. 1958;66(42):957-8.
14. Sabin AB, Feldman HA. Dyes as Microchemical Indicators of a New Immunity Phenomenon Affecting a Protozoon Parasite (Toxoplasma). Science. 1948;108(2815):660-3.
15. Frenkel JK, Dubey JP, Miller NL. Toxoplasma gondii in cats: fecal stages identified as coccidian oocysts. Science. 1970;167(3919):893-6.
16. Magnusson JH. Toxoplasmic Encephalitis in a Child Infected in Utero. Acta Psych Neurol Su. 1947(46):193-203.
17. Boeson I. Et tilfælde af kongenit toxoplasmose. Ugeskr Laeger. 1948;110(226).
18. Standal B, Kass E. Congenitt toxoplasmose [Congenital toxoplasmosis; the first case diagnosed in Norway]. Tidsskr Nor Laegeforen. 1952;72(8):235-7.
19. Stray-Pedersen B, Lorentzen-Styr AM. Epidemiological aspects of Toxoplasma infections among women in Norway. Acta Obstet Gynecol Scand. 1980;59(4):323-6.
20. Stray-Pedersen B, Lorentzen-Styr AM. Uterine toxoplasma infections and repeated abortions. Am J Obstet Gynecol. 1977;128(7):716-21.
21. Stray-Pedersen B. Infants potentially at risk for congenital toxoplasmosis: a prospective study. Am J Dis Child. 1980;134(7):638-42.
22. Stray-Pedersen B. A prospective study of acquired toxoplasmosis among 8,043 pregnant women in the Oslo area. Am J Obstet Gynecol. 1980;136(3):399-406.

23. Jenum PA, Kapperud G, Stray-Pedersen B, Melby KK, Eskild A, Eng J. Prevalence of Toxoplasma gondii specific immunoglobulin G antibodies among pregnant women in Norway. Epidemiol Infect. 1998;120(1):87-92.
24. Jenum PA, Stray-Pedersen B, Melby KK, Kapperud G, Whitelaw A, Eskild A, et al. Incidence of Toxoplasma gondii infection in 35,940 pregnant women in Norway and pregnancy outcome for infected women. J Clin Microbiol. 1998;36(10):2900-6.
25. Jenum PA, Stray-Pedersen B. Development of specific immunoglobulins G, M, and A following primary Toxoplasma gondii infection in pregnant women. J Clin Microbiol. 1998;36(10):2907-13.
26. Frenkel JK, Dubey JP. Toxoplasmosis and its prevention in cats and man. J Infect Dis. 1972;126(6):664-73.
27. Goldman M, Carver RK, Sulzer AJ. Reproduction of Toxoplasma gondii by internal budding. J Parasitol. 1958;44(2):161-71.
28. Lund E, Lycke E, Sourander P. A cinematographic study of Toxoplasma gondii in cell cultures. Br J Exp Pathol. 1961;42:357-62.
29. Jacot D, Daher W, Soldati-Favre D. Toxoplasma gondii myosin F, an essential motor for centrosomes positioning and apicoplast inheritance. EMBO J. 2013;32(12):1702-16.
30. Frenkel JK. Pathology and pathogenesis of congenital toxoplasmosis. Bull N Y Acad Med. 1974;50(2):182-91.
31. Weiss LM, Kim K. Toxoplasma gondii : the model apicomplexan - perspectives and methods. 2nd edition. ed. Amsterdam: Elsevier/AP; 2014. 1085 p.
32. Howe DK, Sibley LD. Toxoplasma gondii comprises three clonal lineages: correlation of parasite genotype with human disease. J Infect Dis. 1995;172(6):1561-6.
33. Sibley LD, Ajioka JW. Population structure of Toxoplasma gondii: clonal expansion driven by infrequent recombination and selective sweeps. Annu Rev Microbiol. 2008;62:329-51.
34. Stray-Pedersen B. Baillière's clinical obstetrics and gynaecology. London: Baillière Tindall; 1993. p. 107-37.
35. Ades AE, Parker S, Gilbert R, Tookey PA, Berry T, Hjelm M, et al. Maternal prevalence of Toxoplasma antibody based on anonymous neonatal serosurvey: a geographical analysis. Epidemiol Infect. 1993;110(1):127-33.
36. Pappas G, Roussos N, Falagas ME. Toxoplasmosis snapshots: global status of Toxoplasma gondii seroprevalence and implications for pregnancy and congenital toxoplasmosis. Int J Parasitol. 2009;39(12):1385-94.
37. Kapperud G, Jenum PA, Stray-Pedersen B, Melby KK, Eskild A, Eng J. Risk factors for Toxoplasma gondii infection in pregnancy. Results of a prospective case-control study in Norway. Am J Epidemiol. 1996;144(4):405-12.
38. Cook AJ, Gilbert RE, Buffolano W, Zufferey J, Petersen E, Jenum PA, et al. Sources of toxoplasma infection in pregnant women: European multicentre case-control study. European Research Network on Congenital Toxoplasmosis. BMJ. 2000;321(7254):142-7.
39. Berger F, Goulet V, Le Strat Y, Desenclos JC. Toxoplasmosis among pregnant women in France: risk factors and change of prevalence between 1995 and 2003. Rev Epidemiol Sante Publique. 2009;57(4):241-8.
40. Birgisdottir A, Asbjornsdottir H, Cook E, Gislason D, Jansson C, Olafsson I, et al. Seroprevalence of Toxoplasma gondii in Sweden, Estonia and Iceland. Scand J Infect Dis. 2006;38(8):625-31.
41. Benard A, Petersen E, Salamon R, Chene G, Gilbert R, Salmi LR. Survey of European programmes for the epidemiological surveillance of congenital toxoplasmosis. Euro Surveill. 2008;13(15).
42. Forsgren M, Gille E, Ljungstrom I, Nokes DJ. Toxoplasma gondii antibodies in pregnant women in Stockholm in 1969, 1979, and 1987. Lancet. 1991;337(8754):1413-4.

43. Petersson K, Stray-Pedersen B, Malm G, Forsgren M, Evengard B. Seroprevalence of Toxoplasma gondii among pregnant women in Sweden. Acta Obstet Gynecol Scand. 2000;79(10):824-9.
44. Lebech M, Larsen SO, Petersen E. [Occurrence of toxoplasmosis in pregnant women in Denmark. A study of 5.402 pregnant women]. Ugeskr Laeger. 1995;157(38):5242-5.
45. Lappalainen M, Koskela P, Hedman K, Teramo K, Ammala P, Hiilesmaa V, et al. Incidence of primary toxoplasma infections during pregnancy in southern Finland: a prospective cohort study. Scand J Infect Dis. 1992;24(1):97-104.
46. Vaage L, Midtvedt T. Epidemiological aspects of toxoplasmosis. II. The prevalence of positive toxoplasmin reactions in naval recruits from different parts of Norway. Scand J Infect Dis. 1975;7(3):218-21.
47. Harboe A. Toxoplasma dye test titers of 1600 blood donors in Oslo. Acta Pathol Microbiol Scand Suppl. 1952;93:325-31.
48. Langset M, Midtvedt T, Omland T. The frequency of positive dye test in blind children. Acta Ophthalmol (Copenh). 1970;48(1):67-70.
49. Stray-Pedersen B, Lorentzen-Styr AM. The prevalence of toxoplasma antibodies among 11,736 pregnant women in Norway. Scand J Infect Dis. 1979;11(2):159-65.
50. Bjerke SE, Vangen S, Holter E, Stray-Pedersen B. Infectious immune status in an obstetric population of Pakistani immigrants in Norway. Scand J Public Health. 2011;39(5):464-70.
51. Patton S, Zimmerman J, Roberts T, Faulkner C, Diderrich V, Assadi-Rad A, et al. Seroprevalence of Toxoplasma gondii in hogs in the National Animal Health Monitoring System (NAHMS). J Eukaryot Microbiol. 1996;43(5):121.
52. Kijlstra A, Jongert E. Control of the risk of human toxoplasmosis transmitted by meat. Int J Parasitol. 2008;38(12):1359-70.
53. Foulon W, Naessens A, Ho-Yen D. Prevention of congenital toxoplasmosis. J Perinat Med. 2000;28(5):337-45.
54. Granseth T. Norsk turisme. Oslo: Statistics Norway; 2012. p. 31-42.
55. This is Norway. What the figures say. Oslo: Statistics Norway; 2009. 45 p.
56. Evengard B, Petersson K, Engman ML, Wiklund S, Ivarsson SA, Tear-Fahnehjelm K, et al. Low incidence of toxoplasma infection during pregnancy and in newborns in Sweden. Epidemiol Infect. 2001;127(1):121-7.
57. Buffolano W, Gilbert RE, Holland FJ, Fratta D, Palumbo F, Ades AE. Risk factors for recent toxoplasma infection in pregnant women in Naples. Epidemiol Infect. 1996;116(3):347-51.
58. Bobic B, Jevremovic I, Marinkovic J, Sibalic D, Djurkovic-Djakovic O. Risk factors for Toxoplasma infection in a reproductive age female population in the area of Belgrade, Yugoslavia. Eur J Epidemiol. 1998;14(6):605-10.
59. Boyer KM, Holfels E, Roizen N, Swisher C, Mack D, Remington J, et al. Risk factors for Toxoplasma gondii infection in mothers of infants with congenital toxoplasmosis: Implications for prenatal management and screening. Am J Obstet Gynecol. 2005;192(2):564-71.
60. Petersen E, Vesco G, Villari S, Buffolano W. What do we know about risk factors for infection in humans with Toxoplasma gondii and how can we prevent infections? Zoonoses and public health. 2010;57(1):8-17.
61. Siegel SE, Lunde MN, Gelderman AH, Halterman RH, Brown JA, Levine AS, et al. Transmission of toxoplasmosis by leukocyte transfusion. Blood. 1971;37(4):388-94.
62. Neu HC. Toxoplasmosis transmitted at autopsy. JAMA. 1967;202(8):844-5.
63. Montoya JG, Liesenfeld O. Toxoplasmosis. Lancet. 2004;363(9425):1965-76.
64. Thalhammer O. [Prenatal incubation period (author's transl)]. Padiatr Padol. 1972;7(1):14-9.
65. Daffos F, Forestier F, Capella-Pavlovsky M, Thulliez P, Aufrant C, Valenti D, et al. Prenatal management of 746 pregnancies at risk for congenital toxoplasmosis. N Engl J Med. 1988;318(5):271-5.

66. Dunn D, Wallon M, Peyron F, Petersen E, Peckham C, Gilbert R. Mother-to-child transmission of toxoplasmosis: risk estimates for clinical counselling. Lancet. 1999;353(9167):1829-33.
67. Tenhunen A. Glandular Toxoplasmosis. Occurence of the disease in Finland Acta Pathol Microbiol Scand Suppl. 1964;172:Suppl 172:1-72.
68. Siim JC, Nissen NI. Toxoplasmosis acquisita lymphonodosa in a 62-year-old woman; isolation of Toxoplasma gondli from lymph node and muscle biopsies. Acta Pathol Microbiol Scand. 1958;43(3):298-304.
69. Beverley JK, Beattie CP. Glandular toxoplasmosis; a survey of 30 cases. Lancet. 1958;2(7043):379-84.
70. Grigg ME, Dubey JP, Nussenblatt RB. Ocular toxoplasmosis: lessons from Brazil. Am J Ophthalmol. 2015;159(6):999-1001.
71. Luft BJ, Remington JS. AIDS commentary. Toxoplasmic encephalitis. J Infect Dis. 1988;157(1):1-6.
72. Luft BJ, Remington JS. Toxoplasmic encephalitis in AIDS. Clin Infect Dis. 1992;15(2):211-22.
73. Eichenwald HF. Congenital Toxoplasmosis - a Study of 150 Cases. Ama Journal of Diseases of Children. 1957;94(4):411-2.
74. Diebler C, Dusser A, Dulac O. Congenital toxoplasmosis. Clinical and neuroradiological evaluation of the cerebral lesions. Neuroradiology. 1985;27(2):125-30.
75. di Carlo P, Romano A, Casuccio A, Cillino S, Schimmenti MG, Mancuso G, et al. Investigation and management of Toxoplasma gondii infection in pregnancy and infancy: a prospective study. Acta Pharmacol Sin. 2011;32(8):1063-70.
76. Herman-Sucharska I, Bekiesinska-Figatowska M, Urbanik A. Fetal central nervous system malformations on MR images. Brain Dev. 2009;31(3):185-99.
77. Wallon M, Peyron F, Cornu C, Vinault S, Abrahamowicz M, Kopp CB, et al. Congenital toxoplasma infection: monthly prenatal screening decreases transmission rate and improves clinical outcome at age 3 years. Clin Infect Dis. 2013;56(9):1223-31.
78. Berrebi A, Assouline C, Bessieres MH, Lathiere M, Cassaing S, Minville V, et al. Long-term outcome of children with congenital toxoplasmosis. Am J Obstet Gynecol. 2010;203(6):552.e1-6.
79. Prusa AR, Kasper DC, Pollak A, Olischar M, Gleiss A, Hayde M. Amniocentesis for the detection of congenital toxoplasmosis: results from the nationwide Austrian prenatal screening program. Clin Microbiol Infect. 2015;21(2):191.e1-8.
80. Cortina-Borja M, Tan HK, Wallon M, Paul M, Prusa A, Buffolano W, et al. Prenatal treatment for serious neurological sequelae of congenital toxoplasmosis: an observational prospective cohort study. PLoS Med. 2010;7(10).
81. Wallon M, Garweg JG, Abrahamowicz M, Cornu C, Vinault S, Quantin C, et al. Ophthalmic outcomes of congenital toxoplasmosis followed until adolescence. Pediatrics. 2014;133(3):e601-8.
82. Faucher B, Garcia-Meric P, Franck J, Minodier P, Francois P, Gonnet S, et al. Long-term ocular outcome in congenital toxoplasmosis: a prospective cohort of treated children. J Infect. 2012;64(1):104-9.
83. Pedersen MG, Mortensen PB, Norgaard-Pedersen B, Postolache TT. Toxoplasma gondii infection and self-directed violence in mothers. Arch Gen Psychiatry. 2012;69(11):1123-30.
84. Pedersen MG, Stevens H, Pedersen CB, Norgaard-Pedersen B, Mortensen PB. Toxoplasma infection and later development of schizophrenia in mothers. Am J Psychiatry. 2011;168(8):814-21.
85. Hayes K, Billson FA, Jack I, Sanderson L, Rogers JG, Greer CH, et al. Cell culture isolation of Toxoplasma gondii from an infant with unusual ocular features. Med J Aust. 1973;1(26):1297-9.
86. Chang CH, Stulberg C, Bollinger RO, Walker R, Brough AJ. Isolation of Toxoplasma gondii in tissue culture. J Pediatr. 1972;81(4):790-1.

87. Derouin F, Mazeron MC, Garin YJ. Comparative study of tissue culture and mouse inoculation methods for demonstration of Toxoplasma gondii. J Clin Microbiol. 1987;25(9):1597-600.
88. Hedman K, Lappalainen M, Seppaia I, Makela O. Recent primary toxoplasma infection indicated by a low avidity of specific IgG. J Infect Dis. 1989;159(4):736-40.
89. Jenum PA, Stray-Pedersen B, Gundersen AG. Improved diagnosis of primary Toxoplasma gondii infection in early pregnancy by determination of antitoxoplasma immunoglobulin G avidity. J Clin Microbiol. 1997;35(8):1972-7.
90. Lebech M, Joynson DH, Seitz HM, Thulliez P, Gilbert RE, Dutton GN, et al. Classification system and case definitions of Toxoplasma gondii infection in immunocompetent pregnant women and their congenitally infected offspring. European Research Network on Congenital Toxoplasmosis. Eur J Clin Microbiol Infect Dis. 1996;15(10):799-805.
91. Liesenfeld O, Montoya JG, Kinney S, Press C, Remington JS. Effect of testing for IgG avidity in the diagnosis of Toxoplasma gondii infection in pregnant women: experience in a US reference laboratory. J Infect Dis. 2001;183(8):1248-53.
92. Ho-yen DO. Clinical features. In: Ho-yen DO, Joss AWL, editors. Human toxoplasmosis. Oxford ; New York: Oxford University Press; 1992. p. 56-78.
93. Mazumder P, Chuang HY, Wentz MW, Wiedbrauk DL. Latex agglutination test for detection of antibodies to Toxoplasma gondii. J Clin Microbiol. 1988;26(11):2444-6.
94. Dannemann BR, Vaughan WC, Thulliez P, Remington JS. Differential agglutination test for diagnosis of recently acquired infection with Toxoplasma gondii. J Clin Microbiol. 1990;28(9):1928-33.
95. Balsari A, Poli G, Molina V, Dovis M, Petruzzelli E, Boniolo A, et al. ELISA for toxoplasma antibody detection: a comparison with other serodiagnostic tests. J Clin Pathol. 1980;33(7):640-3.
96. Lin TM, Halbert SP, O'Connor GR. Standardized quantitative enzyme-linked immunoassay for antibodies to Toxoplasma gondii. J Clin Microbiol. 1980;11(6):675-81.
97. Murray PR, Rosenthal KS, Pfaller MA. Medical microbiology. 7th Edition ed. Philadelphia: Elsevier/Saunders; 2013. x, 874 p.
98. Plantaz D, Goullier A, Jouk PS, Bost M. [Value of the immunosorbent agglutination assay (ISAGA) in the early diagnosis of congenital toxoplasmosis]. Pediatrie. 1987;42(5):387-91.
99. Filice G, Carnevale G, Meroni V, Caligaris S, Ottoboni P, Milano F, et al. IgM-IFA, IgM-ELISA, DS-IgM-ELISA, IgM-ISAGA, performed on whole serum and IgM fractions, for detection of IgM anti-toxoplasma antibodies during pregnancy. Boll Ist Sieroter Milan. 1986;65(2):131-7.
100. Lappalainen M, Hedman K. Serodiagnosis of toxoplasmosis. The impact of measurement of IgG avidity. Ann Ist Super Sanita. 2004;40(1):81-8.
101. Lappalainen M, Koskela P, Koskiniemi M, Ammala P, Hiilesmaa V, Teramo K, et al. Toxoplasmosis acquired during pregnancy: improved serodiagnosis based on avidity of IgG. J Infect Dis. 1993;167(3):691-7.
102. Jenum PA, Holberg-Petersen M, Melby KK, Stray-Pedersen B. Diagnosis of congenital Toxoplasma gondii infection by polymerase chain reaction (PCR) on amniotic fluid samples. The Norwegian experience. APMIS. 1998;106(7):680-6.
103. Abbas AM. Comparative study of methods used for the isolation of Toxoplasma gondii. Bull World Health Organ. 1967;36(2):344-6.
104. Davidson MM. New techniques. In: Ho-yen DO, Joss AWL, editors. Human toxoplasmosis. Oxford ; New York: Oxford University Press; 1992. p. 204-31.
105. Hohlfeld P, Daffos F, Costa JM, Thulliez P, Forestier F, Vidaud M. Prenatal diagnosis of congenital toxoplasmosis with a polymerase-chain-reaction test on amniotic fluid. N Engl J Med. 1994;331(11):695-9.
106. Grover CM, Thulliez P, Remington JS, Boothroyd JC. Rapid prenatal diagnosis of congenital Toxoplasma infection by using polymerase chain reaction and amniotic fluid. J Clin Microbiol. 1990;28(10):2297-301.

107. Wallon M, Franck J, Thulliez P, Huissoud C, Peyron F, Garcia-Meric P, et al. Accuracy of real-time polymerase chain reaction for Toxoplasma gondii in amniotic fluid. Obstet Gynecol. 2010;115(4):727-33.
108. Reischl U, Bretagne S, Kruger D, Ernault P, Costa JM. Comparison of two DNA targets for the diagnosis of Toxoplasmosis by real-time PCR using fluorescence resonance energy transfer hybridization probes. BMC Infect Dis. 2003;3:7.
109. de Oliveira Azevedo CT, do Brasil PE, Guida L, Lopes Moreira ME. Performance of Polymerase Chain Reaction Analysis of the Amniotic Fluid of Pregnant Women for Diagnosis of Congenital Toxoplasmosis: A Systematic Review and Meta-Analysis. PLoS One. 2016;11(4):e0149938.
110. Homan WL, Vercammen M, De Braekeleer J, Verschueren H. Identification of a 200- to 300-fold repetitive 529 bp DNA fragment in Toxoplasma gondii, and its use for diagnostic and quantitative PCR. Int J Parasitol. 2000;30(1):69-75.
111. Belaz S, Gangneux J-P, Dupretz P, Guiguen C, Robert-Gangneux F. A 10-Year Retrospective Comparison of Two Target Sequences, REP-529 and B1, for Toxoplasma gondii Detection by Quantitative PCR. J Clin Microbiol. 2015;53(4):1294-300.
112. Romand S, Wallon M, Franck J, Thulliez P, Peyron F, Dumon H. Prenatal diagnosis using polymerase chain reaction on amniotic fluid for congenital toxoplasmosis. Obstet Gynecol. 2001;97(2):296-300.
113. Hohlfeld P, MacAleese J, Capella-Pavlovski M, Giovangrandi Y, Thulliez P, Forestier F, et al. Fetal toxoplasmosis: ultrasonographic signs. Ultrasound Obstet Gynecol. 1991;1(4):241-4.
114. Jacobson CB, Barter RH. Intrauterine diagnosis and management of genetic defects. Am J Obstet Gynecol. 1967;99(6):796-807.
115. Thulliez P, Remington JS, Santoro F, Ovlaque G, Sharma S, Desmonts G. [A new agglutination reaction for the diagnosis of the developmental stage of acquired toxoplasmosis]. Pathol Biol (Paris). 1986;34(3):173-7.
116. Athanasiadis AP, Pantazis K, Goulis DG, Chatzigeorgiou K, Vaitsi V, Assimakopoulos E, et al. Comparison between 20G and 22G needle for second trimester amniocentesis in terms of technical aspects and short-term complications. Prenat Diagn. 2009;29(8):761-5.
117. Tabor A, Alfirevic Z. Update on procedure-related risks for prenatal diagnosis techniques. Fetal Diagn Ther. 2010;27(1):1-7.
118. Tabor A, Philip J, Madsen M, Bang J, Obel EB, Norgaard-Pedersen B. Randomised controlled trial of genetic amniocentesis in 4606 low-risk women. Lancet. 1986;1(8493):1287-93.
119. Wulff CB, Gerds TA, Rode L, Ekelund CK, Petersen OB, Tabor A. Risk of fetal loss associated with invasive testing following combined first-trimester screening for Down syndrome: a national cohort of 147 987 singleton pregnancies. Ultrasound Obstet Gynecol. 2016;47(1):38-44.
120. Eyles DE, Coleman N. The effect of sulfadimetine, sulfisoxazole, and sulfapyrazine against mouse toxoplasmosis. Antibiotics & chemotherapy (Northfield, Ill). 1955;5(10):525-8.
121. Mas Bakal P, Veld NiT. Postponed spiramycin treatment of acute toxoplasmosis in white mice. Trop Geogr Med. 1965;17(3):254-60.
122. Couvreur J, Thulliez P, Daffos F, Aufrant C, Bompard Y, Gesquiere A, et al. In utero treatment of toxoplasmic fetopathy with the combination pyrimethamine-sulfadiazine. Fetal Diagn Ther. 1993;8(1):45-50.
123. Sheffield HG, Melton ML. Effect of pyrimethamine and sulfadiazine on the fine structure and multiplication of Toxoplasma gondii in cell cultures. J Parasitol. 1975;61(4):704-12.
124. Ambroise-Thomas P, Petersen E. Congenital toxoplasmosis : scientific background, clinical management, and control. New York: Springer; 2000. xi, 324 p.
125. Araujo FG, Huskinson J, Remington JS. Remarkable in vitro and in vivo activities of the hydroxynaphthoquinone 566C80 against tachyzoites and tissue cysts of Toxoplasma gondii. Antimicrob Agents Chemother. 1991;35(2):293-9.

126. McLeod R, Mack D, Foss R, Boyer K, Withers S, Levin S, et al. Levels of pyrimethamine in sera and cerebrospinal and ventricular fluids from infants treated for congenital toxoplasmosis. Toxoplasmosis Study Group. Antimicrob Agents Chemother. 1992;36(5):1040-8.
127. Frenkel JK, Hitchings GH. Relative reversal by vitamins (rho-aminobenzoic, folic, and folinic acids) of the effects of sulfadiazine and pyrimethamine on Toxoplasma, mouse and man. Antibiotics & chemotherapy (Northfield, Ill). 1957;7(12):630-8.
128. Desmonts G, Couvreur J. Toxoplasmosis in pregnancy and its transmission to the fetus. Bull N Y Acad Med. 1974;50(2):146-59.
129. Kernbaum S. [Spiramycin ; therapeutic value in humans. (author's transl)]. Sem Hop. 1982;58(5):289-97.
130. Leport C, Vilde JL, Katlama C, Regnier B, Matheron S, Saimot AG. Failure of spiramycin to prevent neurotoxoplasmosis in immunosuppressed patients. JAMA. 1986;255(17):2290.
131. Forestier F, Daffos F, Rainaut M, Desnottes JF, Gaschard JC. [Fetomaternal therapeutic follow-up of spiramycin during pregnancy]. Arch Fr Pediatr. 1987;44(7):539-44.
132. Ramsey PS, Vaules MB, Vasdev GM, Andrews WW, Ramin KD. Maternal and transplacental pharmacokinetics of azithromycin. Am J Obstet Gynecol. 2003;188(3):714-8.
133. Cooper WO, Hernandez-Diaz S, Arbogast PG, Dudley JA, Dyer SM, Gideon PS, et al. Antibiotics potentially used in response to bioterrorism and the risk of major congenital malformations. Paediatr Perinat Epidemiol. 2009;23(1):18-28.
134. Bar-Oz B, Diav-Citrin O, Shechtman S, Tellem R, Arnon J, Francetic I, et al. Pregnancy outcome after gestational exposure to the new macrolides: a prospective multi-center observational study. Eur J Obstet Gynecol Reprod Biol. 2008;141(1):31-4.
135. McLeod R, Kieffer F, Sautter M, Hosten T, Pelloux H. Why prevent, diagnose and treat congenital toxoplasmosis? Mem Inst Oswaldo Cruz. 2009;104(2):320-44.
136. Foulon W, Villena I, Stray-Pedersen B, Decoster A, Lappalainen M, Pinon JM, et al. Treatment of toxoplasmosis during pregnancy: a multicenter study of impact on fetal transmission and children's sequelae at age 1 year. Am J Obstet Gynecol. 1999;180(2 Pt 1):410-5.
137. Schoondermark-van de Ven E, Galama J, Vree T, Camps W, Baars I, Eskes T, et al. Study of treatment of congenital Toxoplasma gondii infection in rhesus monkeys with pyrimethamine and sulfadiazine. Antimicrob Agents Chemother. 1995;39(1):137-44.
138. Gratzl R, Sodeck G, Platzer P, Jager W, Graf J, Pollak A, et al. Treatment of toxoplasmosis in pregnancy: concentrations of spiramycin and neospiramycin in maternal serum and amniotic fluid. Eur J Clin Microbiol Infect Dis. 2002;21(1):12-6.
139. Thiebaut R, Leproust S, Chene G, Gilbert R. Effectiveness of prenatal treatment for congenital toxoplasmosis: a meta-analysis of individual patients' data. Lancet. 2007;369(9556):115-22.
140. Rivera Fernandez N, Mondragon Castelan M, Gonzalez Pozos S, Ramirez Flores CJ, Mondragon Gonzalez R, Gomez de Leon CT, et al. A new type of quinoxalinone derivatives affects viability, invasion, and intracellular growth of Toxoplasma gondii tachyzoites in vitro. Parasitol Res. 2016;115(5):2081-96.
141. Lu G, Zhou A, Meng M, Wang L, Han Y, Guo J, et al. Alpha-galactosylceramide enhances protective immunity induced by DNA vaccine of the SAG5D gene of Toxoplasma gondii. BMC Infect Dis. 2014;14:3862.
142. Sepulveda-Arias JC, Veloza LA, Mantilla-Muriel LE. Anti-Toxoplasma activity of natural products: a review. Recent patents on anti-infective drug discovery. 2014;9(3):186-94.
143. Desmonts G, Naot Y, Remington JS. Immunoglobulin M-immunosorbent agglutination assay for diagnosis of infectious diseases: diagnosis of acute congenital and acquired Toxoplasma infections. J Clin Microbiol. 1981;14(5):486-91.
144. Desmonts G, Remington JS. Direct agglutination test for diagnosis of Toxoplasma infection: method for increasing sensitivity and specificity. J Clin Microbiol. 1980;11(6):562-8.
145. van Loon AM, van der Logt JT, Heessen FW, van der Veen J. Enzyme-linked immunosorbent assay that uses labeled antigen for detection of immunoglobulin M and A antibodies in

toxoplasmosis: comparison with indirect immunofluorescence and double-sandwich enzyme-linked immunosorbent assay. J Clin Microbiol. 1983;17(6):997-1004.
146. Cesbron JY, Capron A, Ovlaque G, Santoro F. Use of a monoclonal antibody in a double-sandwich ELISA for detection of IgM antibodies to Toxoplasma gondii major surface protein (P30). J Immunol Methods. 1985;83(1):151-8.
147. Stray-Pedersen B, Jenum P. Economic evaluation of preventive programmes against congenital toxoplasmosis. Scand J Infect Dis Suppl. 1992;84:86-96.
148. Hareide B. [Screening of pregnant women for toxoplasma infection]. Tidsskr Nor Laegeforen. 1997;117(3):327-8.
149. Roser D, Nielsen HV, Petersen E, Saugmann-Jensen P, Norgaard-Pedersen B. Congenital toxoplasmosis--a report on the Danish neonatal screening programme 1999-2007. J Inherit Metab Dis. 2010;33(Suppl 2):S241-7.
150. Barlinn R, Vainio K, Samdal HH, Nordbo SA, Nokleby H, Dudman SG. Susceptibility to cytomegalovirus, parvovirus B19 and age-dependent differences in levels of rubella antibodies among pregnant women. J Med Virol. 2014;86(5):820-6.
151. Vink J, Wapner R, D'Alton ME. Prenatal diagnosis in twin gestations. Semin Perinatol. 2012;36(3):169-74.
152. Lov om vern mot smittsomme sykdommer, LOV-1994-08-05-55, § 3,7. Sect. 3 (1995).
153. Grimes DA, Schulz KF. Descriptive studies: what they can and cannot do. Lancet. 2002;359(9301):145-9.
154. Rothman KJ, Greenland S, Lash TL. Modern epidemiology. 3rd ed. Philadelphia: Wolters Kluwer Health/Lippincott Williams & Wilkins; 2008. x, 758 p.
155. Laake P HA, Thelle D.S, Veierød M.B. Epidemiologiske og kliniske forskningsmetoder. Veierod MB, editor: Gyldendal; 2007. 551 p.
156. Findal G, Barlinn R, Sandven I, Stray-Pedersen B, Nordbo SA, Samdal HH, et al. Toxoplasma prevalence among pregnant women in Norway: a cross-sectional study. APMIS. 2015;123(4):321-5.
157. Evans R, Ho-Yen DO. Evidence-based diagnosis of toxoplasma infection. Eur J Clin Microbiol Infect Dis. 2000;19(11):829-33.
158. Barnhart HX, Haber MJ, Lin LI. An overview on assessing agreement with continuous measurements. J Biopharm Stat. 2007;17(4):529-69.
159. Grimes DA, Schulz KF. Bias and causal associations in observational research. Lancet. 2002;359(9302):248-52.
160. Barra M, Lindstrom JC, Adams SS, Augestad LA. Seasonally adjusted birth frequencies follow the Poisson distribution. Tidsskr Nor Laegeforen. 2015;135(23-24):2154-8.
161. Kleinbaum DG, Kupper LL, Morgenstern H. Epidemiologic research : principles and quantitative methods. Belmont, Calif.: Lifetime Learning Publications; 1982. xix, 529 p.
162. Flatt A, Shetty N. Seroprevalence and risk factors for toxoplasmosis among antenatal women in London: a re-examination of risk in an ethnically diverse population. Eur J Public Health. 2013;23(4):648-52.
163. Logar J, Soba B, Premru-Srsen T, Novak-Antolic Z. Seasonal variations in acute toxoplasmosis in pregnant women in Slovenia. Clin Microbiol Infect. 2005;11(10):852-5.
164. Meenken C, Rothova A, Kijlstra A, Oosting J. Seasonal variation in congenital toxoplasmosis. Br J Ophthalmol. 1991;75(10):639.
165. Olsen MA, Root PP. Comparison of four different immunoassays for detection of Toxoplasma-specific immunoglobulin G. Diagn Microbiol Infect Dis. 1994;19(1):19-24.
166. Hofgartner WT, Swanzy SR, Bacina RM, Condon J, Gupta M, Matlock PE, et al. Detection of immunoglobulin G (IgG) and IgM antibodies to Toxoplasma gondii: evaluation of four commercial immunoassay systems. J Clin Microbiol. 1997;35(12):3313-5.
167. Sedgwick P. Retrospective cohort studies: advantages and disadvantages. Bmj-Brit Med J. 2014;348.

168. Fouwels AJ, Bredie SJ, Wollersheim H, Schippers GM. A retrospective cohort study on lifestyle habits of cardiovascular patients: how informative are medical records? BMC Health Serv Res. 2009;9:59.
169. Burns TL, Moll PP, Rost CA, Lauer RM. Mothers remember birthweights of adolescent children: the Muscatine Ponderosity Family Study. Int J Epidemiol. 1987;16(4):550-5.
170. Lefevre-Pettazzoni M, Bissery A, Wallon M, Cozon G, Peyron F, Rabilloud M. Impact of spiramycin treatment and gestational age on maturation of Toxoplasma gondii immunoglobulin G avidity in pregnant women. Clin Vaccine Immunol. 2007;14(3):239-43.
171. Paul M. Immunoglobulin G avidity in diagnosis of toxoplasmic lymphadenopathy and ocular toxoplasmosis. Clin Diagn Lab Immunol. 1999;6(4):514-8.
172. Murat JB, L'Ollivier C, Fricker Hidalgo H, Franck J, Pelloux H, Piarroux R. Evaluation of the new Elecsys Toxo IgG avidity assay for toxoplasmosis and new insights into the interpretation of avidity results. Clin Vaccine Immunol. 2012;19(11):1838-43.
173. Dette er Kari og Ola : kvinner og menn i Norge [This is Kari and Ola : women and men in Norway]. Oslo: Statistics Norway; 2010. 31 p.
174. Innvandrere og norskfødte med innvandrerforeldre, 1. januar 2013: Statistics Norway; 2013. Available from: http://www.ssb.no/befolkning/statistikker/innvbef/aar/2013-04-25.
175. Altman DG, Royston P. The cost of dichotomising continuous variables. BMJ. 2006;332(7549):1080.
176. Kirkwood BR, Sterne JAC, Kirkwood BR. Essential medical statistics. 2nd ed. Malden, Mass.: Blackwell Science; 2003. x, 501 p.
177. Ellertsen LK, Hetland G, Lovik M. Specific IgE to respiratory allergens and IgG antibodies to Toxoplasma gondii and Streptococcus pneumoniae in Norwegian military recruits. Scand J Immunol. 2008;67(5):496-500.
178. Meerburg BG, Kijlstra A. Changing climate-changing pathogens: Toxoplasma gondii in North-Western Europe. Parasitol Res. 2009;105(1):17-24.
179. McMichael AJ. Extreme weather events and infectious disease outbreaks. Virulence. 2015;6(6):543-7.
180. Jones KE, Patel NG, Levy MA, Storeygard A, Balk D, Gittleman JL, et al. Global trends in emerging infectious diseases. Nature. 2008;451(7181):990-3.
181. Carver S, Mills JN, Parmenter CA, Parmenter RR, Richardson KS, Harris RL, et al. Toward a Mechanistic Understanding of Environmentally Forced Zoonotic Disease Emergence: Sin Nombre Hantavirus. Bioscience. 2015;65(7):651-66.
182. Dubey JP. Toxoplasma gondii oocyst survival under defined temperatures. J Parasitol. 1998;84(4):862-5.
183. van den Hurk B, Tank AK, Lenderink G, Ulden A, Oldenborgh GJ, Katsman C, et al. New climate change scenarios for the Netherlands. Water science and technology : a journal of the International Association on Water Pollution Research. 2007;56(4):27-33.
184. Prestrud KW, Asbakk K, Fuglei E, Mork T, Stien A, Ropstad E, et al. Serosurvey for Toxoplasma gondii in arctic foxes and possible sources of infection in the high Arctic of Svalbard. Vet Parasitol. 2007;150(1-2):6-12.
185. Flori P, Tardy L, Patural H, Bellete B, Varlet MN, Hafid J, et al. Reliability of immunoglobulin G antitoxoplasma avidity test and effects of treatment on avidity indexes of infants and pregnant women. Clin Diagn Lab Immunol. 2004;11(4):669-74.
186. Lefevre-Pettazzoni M, Le Cam S, Wallon M, Peyron F. Delayed maturation of immunoglobulin G avidity: implication for the diagnosis of toxoplasmosis in pregnant women. Eur J Clin Microbiol Infect Dis. 2006;25(11):687-93.
187. Aagaard-Tillery KM, Silver R, Dalton J. Immunology of normal pregnancy. Semin Fetal Neonatal Med. 2006;11(5):279-95.
188. Meroni V, Genco F, Tinelli C, Lanzarini P, Bollani L, Stronati M, et al. Spiramycin treatment of Toxoplasma gondii infection in pregnant women impairs the production and the avidity

maturation of T. gondii-specific immunoglobulin G antibodies. Clin Vaccine Immunol. 2009;16(10):1517-20.
189. Alvarado-Esquivel C, Niewiadomski A, Schweickert B, Liesenfeld O. Antiparasitic treatment suppresses production and avidity of Toxoplasma gondii-specific antibodies in a murine model of acute infection*. Eur J Microbiol Immunol (Bp). 2011;1(3):249-55.
190. Sensini A. Toxoplasma gondii infection in pregnancy: opportunities and pitfalls of serological diagnosis. Clin Microbiol Infect. 2006;12(6):504-12.
191. Villard O, Cimon B, L'Ollivier C, Fricker-Hidalgo H, Godineau N, Houze S, et al. Serological diagnosis of Toxoplasma gondii infection: Recommendations from the French National Reference Center for Toxoplasmosis. Diagn Microbiol Infect Dis. 2016;84(1):22-33.
192. Lappalainen M, Koskiniemi M, Hiilesmaa V, Ammala P, Teramo K, Koskela P, et al. Outcome of children after maternal primary Toxoplasma infection during pregnancy with emphasis on avidity of specific IgG. The Study Group. Pediatr Infect Dis J. 1995;14(5):354-61.
193. Prusa AR, Kasper DC, Pollak A, Gleiss A, Waldhoer T, Hayde M. The Austrian Toxoplasmosis Register, 1992-2008. Clin Infect Dis. 2015;60(2):e4-e10.
194. Kuperman AA, Koren O. Antibiotic use during pregnancy: how bad is it? BMC Med. 2016;14(1):91.
195. Kenyon S, Pike K, Jones DR, Brocklehurst P, Marlow N, Salt A, et al. Childhood outcomes after prescription of antibiotics to pregnant women with spontaneous preterm labour: 7-year follow-up of the ORACLE II trial. Lancet. 2008;372(9646):1319-27.
196. Lapin B, Piorkowski J, Ownby D, Freels S, Chavez N, Hernandez E, et al. Relationship between prenatal antibiotic use and asthma in at-risk children. Ann Allergy Asthma Immunol. 2015;114(3):203-7.
197. Helbig A, Kaasen A, Malt UF, Haugen G. Does antenatal maternal psychological distress affect placental circulation in the third trimester? PLoS One. 2013;8(2):e57071.
198. Kemp J, Davenport M, Pernet A. Antenatally diagnosed surgical anomalies: the psychological effect of parental antenatal counseling. J Pediatr Surg. 1998;33(9):1376-9.
199. Kaasen A, Helbig A, Malt UF, Naes T, Skari H, Haugen G. Acute maternal social dysfunction, health perception and psychological distress after ultrasonographic detection of a fetal structural anomaly. BJOG. 2010;117(9):1127-38.
200. Khoshnood B, De Vigan C, Goffinet F, Leroy V. Prenatal screening and diagnosis of congenital toxoplasmosis: a review of safety issues and psychological consequences for women who undergo screening. Prenat Diagn. 2007;27(5):395-403.
201. Berrebi A, Kobuch WE, Bessieres MH, Bloom MC, Rolland M, Sarramon MF, et al. Termination of pregnancy for maternal toxoplasmosis. Lancet. 1994;344(8914):36-9.
202. Cederholm M, Haglund B, Axelsson O. Infant morbidity following amniocentesis and chorionic villus sampling for prenatal karyotyping. BJOG. 2005;112(4):394-402.
203. Tharmaratnam S, Sadek S, Steele EK, Harper MA, Stewart FJ, Nevin J, et al. Early amniocentesis: effect of removing a reduced volume of amniotic fluid on pregnancy outcome. Prenat Diagn. 1998;18(8):773-8.
204. Cebesoy FB, Balat O, Pehlivan S, Kutlar I, Dikensoy E, Ugur MG. Is pregnancy loss after amniocentesis related to the volume of amniotic fluid obtained? Arch Gynecol Obstet. 2009;279(3):357-60.
205. Norwegian Institute of Public Health. Årstabeller for medisinsk fødselsregister 2010. Oslo: Norwegian Institute of Public Health; 2012.
206. Kuppermann M, Gates E, Washington AE. Racial-ethnic differences in prenatal diagnostic test use and outcomes: preferences, socioeconomics, or patient knowledge? Obstet Gynecol. 1996;87(5 Pt 1):675-82.
207. Dormandy E, Michie S, Hooper R, Marteau TM. Low uptake of prenatal screening for Down syndrome in minority ethnic groups and socially deprived groups: a reflection of women's attitudes or a failure to facilitate informed choices? Int J Epidemiol. 2005;34(2):346-52.

208. Olariu TR, Remington JS, McLeod R, Alam A, Montoya JG. Severe congenital toxoplasmosis in the United States: clinical and serologic findings in untreated infants. Pediatr Infect Dis J. 2011;30(12):1056-61.
209. Montoya JG, Remington JS. Management of Toxoplasma gondii infection during pregnancy. Clin Infect Dis. 2008;47(4):554-66.
210. Jeannel D, Costagliola D, Niel G, Hubert B, Danis M. What is known about the prevention of congenital toxoplasmosis? Lancet. 1990;336(8711):359-61.

11 Appendix

Pas.nr:

Til deg som har blitt utredet for toxoplasma infeksjon i svangerskapet ved Oslo Universitetssykehus de siste 20 årene

-Forespørsel om tilleggsinformasjon

Du husker sikkert at dersom en gravid blir smittet av parasitten Toxoplasma gondii, kan infeksjonen overføres til fosteret med større eller mindre senfølger for barnet. Heldigvis er det slik at de fleste fostre ikke blir smittet og heller ikke utvikler skader.

For å gi riktige råd og omsorg til fremtidige gravide utfører vi nå en evalueringsstudie av våre undersøkelsesmetoder og behandlingsopplegg for toxoplasmose på Oslo Universitetssykehus, Rikshospitalet.

Du kontaktes fordi du fikk tilbud om å ta en fostervannsprøve. Hensikten med dette var å finne ut om fosteret ditt var smittet av toxoplasma parasitten eller ikke. Fostervannsprøven ved toxoplasmose er litt annerledes enn andre fostervannsprøver. Vi tar ut mindre fostervann og dette tror vi gir mindre ettervirkninger (abortfare, fostervannslekkasje, for tidlig fødsel). Dette er litt av bakgrunnen for undersøkelsen, men vi mangler informasjon om hvordan det gikk med svangerskapet ditt og disse opplysningene er viktig for oss for å vurdere virkningene og komplikasjonene av våre prosedyrer.

Dersom du opplevde denne utredningen som belastende eller dersom svangerskapet endte på en uønsket eller trist måte, beklager vi om vi bringer fram vonde minner. Din informasjon og dine erfaringer er imidlertid verdifulle for oss.

Denne evalueringsstudien utgår fra Universitetet i Oslo og Kvinne- Barne klinikken ved Oslo Universitetssykehus, Rikshospitalet under ledelse av professor Babill Stray Pedersen, spesialist i kvinnesykdommer og fødselshjelp og ekspert på toxoplasmose i samarbeid med ultralydenheten ved Kvinne-Barne klinikken ved professor Guttorm Haugen og Mikrobiologisk avdeling (Referanselaboratorie forToxoplasmose i Norge) ved professor Pål Jenum.

Hva innebærer evalueringsstudien?
Dersom du ønsker å supplere vår informasjon, ber vi deg besvare spørsmålene på neste side, og returnere papirene i vedlagte svarkonvolutt, sende svaret pr mail el SMS. Har vi ikke mottatt svar, vil lege Gry Findal ringe om to uker for å innhente relevant informasjon.

Hva skjer med informasjonen om deg
All informasjon om deg og ditt barn skal kun brukes slik som beskrevet ovenfor. Alle opplysningene vil bli behandlet uten navn og fødselsnummer eller andre direkte gjenkjennende opplysninger. En kode knytter deg til dine opplysninger og prøvesvar gjennom en navneliste som oppbevares innelåst separat fra annen informasjon. Kun professor Babill Stray-Pedersen og lege Gry Findal har tilgang til denne navnelisten. Det vil ikke være mulig å identifisere deg i resultatene av studien.

- **Frivillig deltakelse**
  Det er frivillig å svare på dette brevet og du behøver ikke gi oss informasjon hvis du ikke ønsker. Klipp ut denne siden med spørsmål og svar og send i vedlagt konvolutt. Brevet vil da kun inneholde et pasientnummer som ikke kan spores tilbake til deg av andre enn Babill Stray-Pedersen og Gry Findal

  Studien er vurdert og godtkjent av Regional etisk komité og Pastentvernombudet ved OUS som en kvalitetssikringsstudie

  Har du spørsmål til studien, kan du kontakte lege Gry Findal (23072648/ 40061559 gryfi@medisin.uio.no) eller professor Babill Stray-Pedersen (23072651/babill.stray-pedersen@medisin.uio.no).

Dette ønsker vi å få informasjon om:

Hvilke dato ble barnet ditt født..........
Hvor mye veide barnet ved fødsel?.........(gram)
Hva var barnets lengde? .... (cm)
Barnets kjønn? Jente..... gutt......
Ble barnet ditt behandlet med antibiotika mot toxoplasmose? ja...... nei........

Hva slags fødselsmåte var det (sett kryss):
-vanlig vaginal fødsel: ......
-fødsel med vakuum eller tang:.......
-Planlagt keisersnitt:......
(grunn til planlagt keisersnitt:...
-Akutt keisersnitt:......

Var barnet friskt ved fødsel?:
Hvis nei; hva feilte det barnet?:...
...

Er barnet friskt i dag?
Hvis nei; hva feiler det barnet?:...
...

Kommentarer/ekstraopplysninger?:..
...
....

Jeg ønsker ikke å gi ytterligere informasjon........

Pasientnummer:

Table 5:Toxoplasma tests in use at the Norwegian Institute of Public Health (FHI) (1993-2002) and Oslo University Hospital (OUS) (2002-2013)

	Test name	Antibody	Test type-technique	Manufacturer	High positive	Positive	Borderline	Negative	Value	Titre steps	Year in use
FHI	Dye -test	IgG	Sabine-Feldmann, antibody measurement	in-house	≥ 300	≥ 6		< 6	Titre steps	3,6,12,25,50, 100,200,400, 800,1600	<2003
FHI	Toxoplasma gondii ISAGA	IgM	Agglutination, microparticle	bioMérieux †		≥ 9-12	6-8	< 6	Index	0-12	<2003
FHI	Platelia IgG	IgG	EIA	Sanofi Diagnostics Pasteur‡		≥ 6		≤ 6	U/mL		<2003
FHI	Platelia IgM	IgM	EIA	Sanofi Diagnostics Pasteur		≥100		≤ 100	% of cut-off		<2003
FHI	Toxo-Screen DA	IgG	Agglutination, microparticle	bioMérieux		≥ 80	40-60	<40	titre steps	40-60-80-160-540-1620-4000-6000-18000-54000-162000	<2003
FHI	Toxo IgG P 30 IgG avidity	IgG	EIA	Sanofi Diagnostics Pasteur + "in-house" reagents		<15% = low	15-20%	>20% = high	index		<2003
FHI	Mouse inoculation										<2003
FHI	Toxoplasma PCR			"in-house"							<2003
OUS	Toxoplasma gondii ISAGA	IgA/IgM	Agglutination, microparticle	bioMérieux		≥ 9-12	6-8	< 6	Index	0-12	2003-2008
OUS	Toxo IgG EIA	IgG	VIDAS	bioMérieux		≥ 8	4,0 -7,9	< 4,0	IU/ml		2003 -2005
OUS	Toxo IgM EIA	IgM	VIDAS	bioMérieux		> 0,65	0,55 -0,65	< 0,55	Index		2003 -2005
OUS	Toxoplasma IgG	IgG	MEIA, AxSym	Abbott¶		≥3,0	2,0 - 2,9	< 2,0	IU/ml		2005 -2009
OUS	Toxoplasma IgM	IgM	MEIA, AxSym	Abbott		> 0,6	0,5-0,6	< 0,5	Index		2005 -2010
OUS	Toxo IgG	IgG	CMIA, Architect	Abbott		< 1,6	*1,6 -2,9	≥ 3,0	IU/ml		>2009
OUS	Toxo IgM	IgM	CMIA, Architect	Abbott		> 0,560	*0,240 -0,560	<0,240	Index		>2010
OUS	Platelia Toxo IgG	IgG	EIA	Bio-Rad‡		≥ 11	*4-11	<4	U/ml		>2003
OUS	Platelia Toxo IgM	IgM	EIA	Bio-Rad		> 120%	*80-120 %	< 80 %	% av cut-off		>2003
OUS	Platelia Toxo IgA	IgA	EIA	Bio-Rad		> 120%	*80-120 %	< 80 %	% av cut-off		>2003
OUS	Toxo IgG P 30 IgG avidity	IgG	EIA	Bio-Rad + "in-house" reagents		<15% = low	15-20%	>20% = high	index		2003-2005
OUS	Toxo IgG avidity	IgG	EIA + supplementary reagents	Bio-Rad		< 0,4=low	0,4-0,5	>0,5 = high	Index		>2005
OUS	Toxoplasma PCR		Real-time	B-1 gene "in-house"							2003-2004
OUS	Toxoplasma PCR		Real-time	Repeat element, bp529, "in-house"							> des 2004

*variable, borderline dependant of CV%, † Marcy l´Etoile, France, ‡Marnes-la-Coquette, France,¶ Wiesbaden, Germany.

12 Errata

Errata list, Gry Findal; Thesis: Toxoplasma infection among pregnant women in Norway; susceptibility, diagnosis and follow-up

Page	Line	Original text	Type	Corrected text (bold)
37	8	Indirect enzyme-immunoassays (EIA) (Platelia Toxo IgG……)	Additional heading	**Immunoassays:** Indirect enzyme-immunoassays (EIA) (Platelia Toxo IgG……)
41	17	…..prevalence levels found in 1974-75 (12.1%) and 1992-93 (10.7%)…..	Correction	…..prevalence levels found in 1974-75 (12.**6**%) and 1992-93 (10.**9**%)…..
56	32	…by Jenum et al. from 1993-1994 in order to ease…	Correction	…by Jenum et al. from 199**2-**199**3** in order to ease…
59	22	….but was still below the high threshold level (Figure12)…	Correction	….but was still below the high threshold level (Figure**10**)…
67	22	…The long duration of the toxoplasma IgG avidity and…..	Addition	…The long duration of the **low** toxoplasma IgG avidity and…..
74	Ref. 80	….cohort study. PLoS Med. 2010;7 (10).	Addition	….cohort study. PLoS Med. 2010;7 (10).**e1000351.**