Epidemiol. Infect. (2001), **127**, 121–127. © 2001 Cambridge University Press
DOI: 10.1017/S0950268801005775 Printed in the United Kingdom

Low incidence of toxoplasma infection during pregnancy and in newborns in Sweden

B. EVENGÅRD[1]*, K. PETERSSON[2], M-L. ENGMAN[3], S. WIKLUND[4], S. A. IVARSSON[4], K. TEÄR-FAHNEHJELM[5], M. FORSGREN[6], R. GILBERT[7] AND G. MALM[3]

Divisions of Clinical Bacteriology, Unit of Parasitology[1], and Virology[6] at Dept Immunology, Microbiology and Pathology, Depts of Obstetrics and Gynaecology[2], Pediatrics[3] and Ophthalmology[5], Karolinska Institutet at Huddinge University Hospital S-141 86 Stockholm, Sweden Dept of Pediatrics[4], Malmö University Hospital, Sweden and Dept of Epidemiology and Biostatistics[7], Institute of Child Health, London, United Kingdom.

(*Accepted 23 March 2001*)

SUMMARY

To estimate the burden of disease due to congenital toxoplasmosis in Sweden the incidence of primary infections during pregnancy and birth prevalence of congenital toxoplasmosis in 40978 children born in two regions in Sweden was determined. Women possibly infected during pregnancy were identified based on: 1, detection of specific IgG based on neonatal screening of the phenylketonuria (PKU) card blood spot followed by retrospective testing of stored prenatal samples to detect women who acquired infection during pregnancy and follow up of their children to 12 months; 2, detection of specific IgM on the PKU blood spot.

The birth prevalence of congenital toxoplasmosis was 0·73/10000 (95% CI 0·15–2·14) (3/40978).

The incidence of primary infection during pregnancy was 5·1/10000 (95% CI 2·6–8·9) susceptible pregnant women. The seroprevalence in the southern part was 25·7% and in the Stockholm area 14·0%.

The incidence of infection during pregnancy was low, as the birth prevalence of congenital toxoplasmosis. Neonatal screening warrants consideration in view of the low cost and feasibility.

INTRODUCTION

The epidemiology of infection with *Toxoplasma gondii* has changed in Sweden since the 1950s. The seroprevalence in pregnant women has decreased in the Stockholm area from around 50% to 14% [1, 2], same thing is also reported from other parts of Europe [3–7]. Within Sweden, the prevalence of infection decreases with latitude, ranging from 26% in the south of Sweden, similar to Denmark [8], to 12% in the north in 1987 [9]. Any burden of disease due to congenital toxoplasmosis, for example neurological or visual impairment due to lesions in the brain and/or retina [10–12] is largely unknown in Sweden. The number of subsequently verified cases of congenital toxoplasmosis has been limited to single reports among the approximately 100000 children born yearly in Sweden. However, reported cases do not represent a true indicator of the real rate as the disease is often asymptomatic. In addition, data on the risk of maternal toxoplasmosis are scanty. An incidence of 4–6/1000 pregnancies was found in a small pilot study from the southern part of Sweden 15 years ago [13]. In

* Author for correspondence.

the Stockholm area in 1987 best estimates of between 0 and 2·7 cases per 1000 pregnancies were obtained by modelling toxoplasma incidence from seroprevalence data [7]. Additionally in 1992–3, 4 seroconverters were found in 3094 pregnant women [2].

The aim of the study was to determine the birth prevalence and risk of clinical signs, symptoms and impairment at 1 year of age due to congenital toxoplasmosis. This would inform rational decisions about preventive measures.

Studies based on women infected after the first prenatal blood sample (taken at 10–12 weeks of gestation) have shown that neonatal detection of IgM on blood spots is 70–80% sensitive [14]. However, sensitivity may be lower for babies born to women infected in the first trimester of pregnancy. We therefore also attempted to identify all women who were possibly infected during pregnancy, in addition to identifying children by neonatal screening. We followed up all children to determine congenital infection status.

POPULATION AND METHODS

After delivery, all mothers of children born in Stockholm County between 1 April 1997 and 31 July 1998 and in Skåne County between 1 May 1997 and 31 July 1998 received written information about the study and consent to participation was sought. If the mother declined this was marked on the phenylketonuria card, (PKU card). Blood spots were collected on filter paper cards between days 3–5 after birth, as part of the routine screening for inborn error of metabolism (e.g. phenylketonuria) and congenital hypothyroidism.

Screening for mothers infected during pregnancy and follow up of their children

Mothers with previous toxoplasma infection were identified by analysis of the eluates from blood spots on PKU cards for toxoplasma IgG antibody activity.

To identify women infected during pregnancy, stored prepartum samples obtained in week 12 (mean, SD 4·61 weeks) from the seropositive mothers were tested for specific IgG and IgM.

The following definitions were used:

(a) definitive infection, seroconversion – a change from undetectable anti-toxoplasma IgG antibodies in the sample drawn during early pregnancy to detectable IgG antibodies after birth; (b) possibly infected; IgG level > 100 IU or demonstrable IgM and an IgG avidity index < 30.

To confirm serological results, women possibly infected during pregnancy and their children were sampled for confirmatory serological analyses (specific IgM/A and IgG tests)

All children born to women who were possibly infected during pregnancy were followed clinically and serologically for 12 months.

Clinical, neurological and ophthalmological assessments and evaluation of developmental milestones were performed at 1, 3, 6, 9, and 12 months. A cranial ultrasound investigation was performed at the first visit and, in children with confirmed congenital toxoplasmosis, computerized tomography of the brain was also performed. A psychometric evaluation using the Griffith developmental scale [15] was performed between 12 and 15 months of age.

The ophthalmological investigations depended on the age of the patients and included external investigation of major ocular malformations, visual acuity assessment with the preferential looking technique, evaluation of ocular motility, cover for the detection of strabismus, evaluation of pupillary reactions to light and indirect ophthalmology monocularly and/or binocularly for the detection of signs of chorioretinitis in the fundi. A hearing assessment was routinely performed at the health care centre at 8 months of age.

Antibody levels were measured between 3 weeks and 3 months, and repeated at 3, 6, 9 and 12 months of age.

The criteria for serodiagnosis of possible toxoplasma infection during pregnancy were based on the definitions of a European Union Concerted Action on Congenital Toxoplasmosis [16] modified by the addition of avidity test [17, 18]. To certify correct identity of samples from the mothers and to exclude other potential hazardous infections, pregnancy sera were tested in parallel against TORCH (toxoplasma, rubella, CMV, herpes simplex virus type common and type 2) antigens.

Neonatal screening

Infected children were identified by detection of toxoplasma IgM in eluates from PKU-cards. If IgM was positive further serum samples were taken from

the child and mother for confirmatory analyses of IgM/IgA. Children with serological markers for toxoplasma infection were followed clinically and serologically to 12 months of age in the same way as the children born to possibly infected mothers.

The criterion for diagnosis of congenital toxoplasmosis was the presence of specific IgG at 12 months of age.

Laboratory assays

Eluates from PKU filter paper cards (Schleicher & Schull no 903, Dassel, Germany): disks were cut out from the PKU cards (diameter 3·2 mm) and analysed for specific IgM antibodies (FEIA, Labsystem, Helsinki) as described [19] and for specific IgG antibodies [20] (EIA, Labsystem, Helsinki).

Maternal sera were analysed for specific antibodies using an automated ELISA (Toxo IgM, Toxo IgG, Abbotts Diagnostics). Sera from the children were analysed for specific IgM and IgG using the same assays and for specific IgM and IgA also ISAGA PLUS IgA/IgM test (Bio-Merieux). Avidity of the IgG antibodies was measured using a commercial assay (Labsystem, Helsinki). The interpretation of the avidity results was according to the manufacturer recommendations: index $> 30\%$ excludes a primary infection within 3 months; an index $< 15\%$ is compatible with an acute infection; 15–30% is an intermediate value that may indicate primary infection during the last 6 months.

PCR for detection of *Toxoplasma gondii* DNA, using a nested technique, was performed in all children born to women with suspected infection during pregnancy using EDTA-blood from the newborn. The DNA was prepared from leucocyte suspension, containing approximately 500 µl lysed EDTA-blood, with the Qiagen Amp Kit (Qiagen, Stockholm, Sweden) and eluted in 100 µl H_2O. 20 µl of the purified DNA were then *T. gondii* B1 gene amplified with a nested PCR method [21] slightly modified from Burg [22]. The nested PCR fragments were separated by ethidium bromide stained agarose gel (4%) electrophoresis and compared to a standard DNA molecular size ladder (100 bp).

The diagnosis of congenital toxoplasmosis was finally established at 12 months of age if the child still had specific IgG antibodies.

This study was approved by the ethical committee at the Karolinska Institutet.

RESULTS

Eluates from 40978 new-borns were analysed; 26885 were from the Stockholm area (65·6%) and 14093 from Skåne (34·4%), representing 97·1% of all newborns during the study period.

Screening for mothers infected during pregnancy and follow up of their children

Specific IgG was found in 3772 cards in Stockholm and 3618 cards in Skåne, giving a seroprevalence of 14·0% in the Stockholm area and 25·7% in Skåne. The results are shown in detail in Figure 1.

In total, 7390 pregnancy sera were requested of mothers with IgG positive children. Of these 30% could not be retrieved due to a health service reorganisation in Skåne. Thus 33589 women were susceptible to toxoplasma infection (i.e. specific IgG negative).

Seroconversion was detected in 12 women. At follow up 3 of the 12 children born to these women had evidence of congenital infection based on specific IgM and IgA antibodies in confirmatory neonatal serum and persistence of IgG at the age of 12 months.

IgG seroconversion but no other serological markers for toxoplasma infection was found in a further six mothers. These sera were, however, not considered further as the antibody profiles in TORCH analysis indicated they had been mislabelled. Children of these six mothers were anyway followed to 12 months but none had any clinical or serological evidence of congenital infection.

The incidence of primary infection with *Toxoplasma gondii* (defined as seroconversion during pregnancy in women giving birth to live children) was 0·51 per 1000 susceptible pregnancies (9 months) (95%CI = 0·26–0·89; based on the Poisson distribution). The overall risk of mother to child transmission was $3/12 = 25\%$.

Another 31 children were enrolled in the follow-up study due to suspicion of infection between conception and the first prenatal sample. Of these 3 were lost to follow-up either due to parental refusal (1), refusal of paediatrician (1) or emigration (1). None of the children lost to follow-up had demonstrable IgM antibodies in the PKU card eluates or clinical signs of toxoplasma infection. All 28 children followed for 12 months became seronegative, thus excluding a congenital infection.

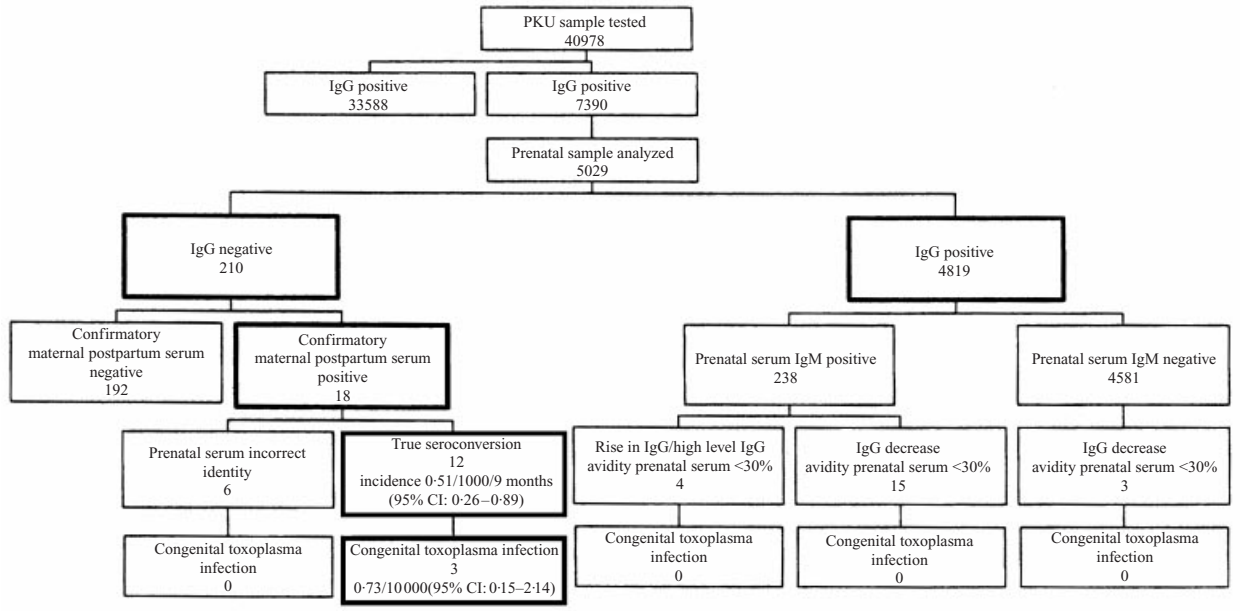

Fig. 1. Toxoplasma IgG screening of pregnant women.

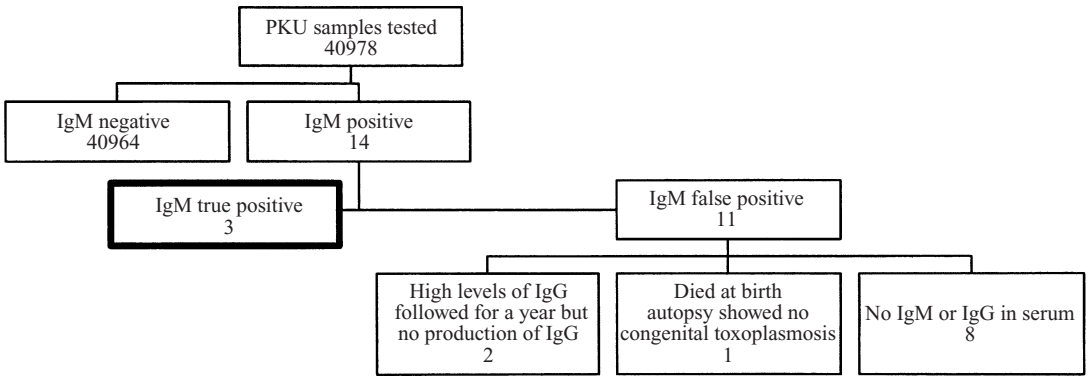

Fig. 2. Toxoplasma IgM screening of newborns.

Neonatal screening

Fourteen children were IgM positive on PKU card screening (Fig. 2). Three of these had congenital infection confirmed at 1 year, and one preterm child died without clinical signs or post mortem findings of congenital toxoplasmosis. In the remaining 10 children the diagnosis of congenital toxoplasmosis was excluded. In 8 of these 10 children neonatal investigation including serum sampling from the child and the mother was required. Confirmatory analyses were negative and further follow up was not indicated. Due to high levels of IgG antibodies in the newborn and the mother, two children were followed until 1 year of age but were not treated.

All three children with congenital toxoplasmosis were born to mothers who had seroconverted during pregnancy and who were identified by the retrospective testing of stored prenatal samples. They were born in gestational week 39 or 40 and received treatment for 1 year [23]. Two of these children had clinical signs of the infection: one had hydrocephalus, intracranial calcifications, a small corneal diameter and chorioretinitis in the right eye; the other had intracranial calcifications [24]. The most severely affected child had a demonstrable toxoplasma DNA in EDTA-blood and specific IgG remained at a high level after completion of treatment.

The birth prevalence of congenital toxoplasmosis in liveborn children was 3/40978 equivalent to 0·73/10000 (95% CI 0·15–2·14).

DISCUSSION

The prevalence of susceptibility to toxoplasma infection in pregnant women was higher than reported in 1982–3. In the southern part of Sweden (Skåne) the seroprevalence fell from 40% to 25·7% and is now

very similar to neighbouring Denmark (27·6%) [14]. As previously reported, the prevalence of seropositivity to toxoplasma infection decreased with latitude, being 14·0% in the Stockholm area. The incidence of infection in susceptible women was 0·51/1000 (95% CI 0·35–1·18) calculated as seroconversions per 1000 pregnancies in susceptible women. Comparison of this figure with other countries is difficult as few studies take account of the time period between the negative and positive test when reporting incidence [6]. Recent estimates of the incidence/1000 susceptible pregnancies are 2·9 (2·48–3·45) in Denmark [14], 0·82 (0·48–1·32) in Norway (based on 11 seroconversions) [25], and 3·4 (2·23–4·86) in Finland [26]. The incidence is similar for Sweden and Norway but significantly higher in Finland and Denmark. These differences may reflect the use of different tests and different cut-offs for test positivity, or genuine regional differences in exposure.

The birth prevalence of congenital toxoplasmosis in this Swedish study is 0·73/10000 but with wide confidence intervals (95% CI 0·15–2·14). The birth prevalence of congenital toxoplasmosis per 10000 live births reported elsewhere in Scandinavia is 3·0 (95% CI 1·98–4·37) in Denmark [14], 3·3 in Norway (95% CI 1·63–5·83) [25], and 2·4 (95% CI 0·7–6·1) in Finland [26]. Although all these studies were performed during a reasonably close time period (Denmark 1992–6, Norway 1992–4, Finland 1988–9), there is a significant difference between the birth prevalence observed in Sweden compared with Denmark ($P = 0{\cdot}01$) and Norway ($P = 0{\cdot}15$) but not Finland ($P = 0{\cdot}11$) (2 sided Fisher's exact test). There may truly be a lower prevalence in Sweden possibly due to different dietary habits and/or latitude. A similar figure to Sweden was reported from New England. This was 0·82/10000 births (95% CI; 0·61–1·07) [27]. Conversely, a recent report from Poznan (Polen), gave a birth prevalence of 4·72/10000 births (2·51–8·08) [28].

False positive results in screening tests results add to the anxiety of parents and to costs and the requirements for specificity are very high. In the present investigation, a setting of a study, the positive predictive value of screening for seroconversion was 6% (12/210 see Fig. 1). In other words, for every 100 women requiring further testing, only 6 truly seroconverted. This figure underestimates the number unnecessarily requiring further testing as we did not undertake retrospective testing of stored samples in mother–child pairs with undetectable IgG on the PKU card. The positive predictive value of a positive neonatal IgM result on PKU card (Fig. 2) was reasonable (3/14, 21%). In our study 11 false positive reactions occurred (0·25/1000). In the majority of cases, serological follow up of the mother and child resolved the diagnostic situation, minimizing the need for unnecessary follow up of the child. Similar results for the positive predictive value of neonatal IgM screening have been reported from other programmes [14, 28]. Previous studies have found an IgM seroprevalence of 50–77% in neonates with congenital toxoplasmosis [29, 30]. In particular, children infected early in pregnancy may have no detectable toxoplasma specific IgM at birth [29]. However, the opportunity for longer treatment of toxoplasma in pregnancy may shorten the duration of IgM response in the fetus. Few data are available on non-treated mother–child pairs. Our data adds only marginal information on the sensitivity of neonatal screening for IgM on PKU cards compared with detection of maternal infection during pregnancy and follow up of the children to 12 months. This is due to the very low prevalence of congenital toxoplasmosis: no more cases were added from the retrospective testing of prenatal samples or reported from the diagnostic laboratories. The low incidence of primary maternal infection in the Swedish pregnant population, means that the uncertain benefit of prenatal screening to a small number of women and children would not outweigh the risk of side effects from invasive diagnosis and treatment and the high cost. However, neonatal screening is clearly feasible and practical. The cost of a neonatal screening based on detection of specific IgM is low, compared to the expenses involved for caring for one severely damaged child. The number of parent falsely alerted for every true case found is minimal.

Neonatal screening has been applied for a decade in the New England area, USA [27] with a similar prevalence of congenital toxoplasmosis to Sweden and was started in 1999 in Denmark. The effectiveness of postnatal treatment in asymptomatic children is not established but is widely believed to be justified [27, 31, 32]. Our results will form the basis for consideration of a Swedish policy on neonatal screening.

ACKNOWLEDGEMENTS

We are grateful for the kind expertise received from A.E.Ades, Institute of Child Health, London when analysing the data.

The study was supported by the Swedish Institute for Infectious Disease Control, the Department of Communicable Disease Control and Prevention at Stockholm County Council, the National Board of Health and Welfare, Health Services Administration, Malmö University hospital, General Maternity hospital foundation (Stiftelsen Allmänna Barnbördshusets Minnesfond), Linnea and Josef Carlssons foundation and Första Majblommans Riksförbund.

We gratefully acknowledge the expert technical assistance of Carina Andersson and Harriet von Post.

REFERENCES

1. Forsgren M, Gille E, Ljungstrom I, Nokes DJ. *Toxoplasma gondii* antibodies in pregnant women in Stockholm in 1969, 1979, and 1987. Lancet 1991; **337**: 1413–4.
2. Evengård B, Lilja G, Capraru T, et al. A retrospective study of seroconversion against *Toxoplasma gondii* during 3000 pregnancies in Stockholm. Scand J Inf Dis 1999; **31**: 127–9.
3. Horion M, Thoumsin H, Senterre J, Lambotte R. 20 years of screening for toxoplasmosis in pregnant women. The Liege experience in 20000 pregnancies. Rev Med Liege 1990; **45**: 492–7.
4. Bornand JE, Piguet JD. Toxoplasma infestation: prevalence, risk of congenital infection and development in Geneva from 1973 to 1987. Schweiz Med Wochenschr 1991; **121**: 21–9.
5. Walker J, Nokes DJ, Jennings R. Longitudinal study of toxoplasma seroprevalence in South Yorkshire. Epidemiol Infect 1992; **108**: 99–106.
6. Ades AE, Nokes DJ. Modeling age- and time-specific incidence from seroprevalence: toxoplasmosis. Am J Epidemiol 1993; **137**: 1022–34.
7. Nokes DJ, Forsgren M, Gille E, Ljungström I. Modelling toxoplasma incidence from longitudinal seroprevalence in Stockholm, Sweden. Parasitology 1993; **107**: 33–40.
8. Lebech M, Larsen SO, Peteresen E. Prevalence, incidence and geographical distribution of Toxoplasma antibodies in pregnant women in Denmark. Scand J Infect Dis 1993; **25**: 751–6.
9. Ljungstrom I, Gille E, Nokes J, Linder E, Forsgren M. Seroepidemiology of *Toxoplasma gondii* among pregnant women in different parts of Sweden. Eur J Epidemiol 1995; **11**: 149–56.
10. Remington JS, Desmonts G. Toxoplasmosis. In: Remington JS, Klein JO, eds. Infectious diseases of the fetus and the newborn infant, 3rd ed. Philadelphia: Saunders 1995′: 140–267.
11. Koppe JG, Rothova A. Congenital toxoplasmosis. A long-term follow-up of twenty years. Internatl Ophthalmol 1989; **13**: 387–90.
12. Wilson CB, Remington JS, Stagnio S, Reynoldas DW. Development of adverse sequelae in children born with subclinical congenital toxoplasma infection. Pediatrics 1980; **66**: 767–74.
13. Ahlfors K, Börjeson M, Huldt G, Forsberg E. Incidence of toxoplasmosis in pregnant women in the city of Malmoe, Sweden. Scand J Infect Dis 1989; **21**: 315–21.
14. Lebech M, Andersen O, Christensen NC, et al. and the Danish Congenital Toxoplasmosis Study group. Feasibility of neonatal screening for toxoplasma infection in the absence of prenatal treatment. Lancet 1999; **353**: 1834–7.
15. Ahlin-Åkerman B, Nordberg I. Griffith's utvecklingsskalor I och II. Manual. Stockholm: Psykologiförlaget 1980.
16. Lebech M, Joynson DHM, Seitz HM et al. Classification system and case definitions of *Toxoplasma gondii* infection in immunocompetent pregnant women and their congenitally infected offspring. Eur J Clin Microbiol Infect Dis 1996; **15**: 799–805.
17. Hedman K, Lappalainen M, Seppälä I, Mäkelä O. Recent primary toxoplasma infection indicated by a low avidity of specific IgG. J Infect Dis 1989; **159**: 736–40.
18. Jenum PA, Stray-Pedersen B, Gundersen A-G. Improved diagnosis of primary *Toxoplasma gondii* infection in early pregnancy by determination of antitoxoplasma immunoglobulin G avidity. J Clin Microbiol 1997; **35**: 1972–77.
19. Eaton RB, Petersen E, Seppanen H, Tuuminen T. Multicenter evaluation of a fluorometric enzyme immunocapture assay to detect toxoplasma-specific immunoglobulin M in dried blood filter paper specimens from newborns. J Clin Microbiol 1996; **34**: 3147–50.
20. Lebech M, Petersen E. Detection of enzyme immunosorbent assay of *Toxoplasma gondii* IgG antibodies in dried blood spots on PKU-filter paper from newborns. Scand J Infect Dis 1995; **27**: 259–63.
21. Wastling JM, Nicoll S, Buxton D. Comparison of two gene amplification methods for the detection of *Toxoplasma gondii* in experimentally infected sheep. J Med Microbiol 1993; **38**: 360–5.
22. Burg JL, Grover MC, Pouletty P, Boothroyd JC. Direct and sensitive detection of a pathogenic protozoan, *Toxoplasma gondii*, by polymerase chain reaction. J Clin Microbiol 1989; **27**: 1787–92.
23. Malm G, Teär Fahnehjelm K, Wiklund S et al. Three children with congenital toxoplasmosis: early report from a Swedish prospective screening study. Acta Paediatr 1999; **88**: 667–70.
24. Teär Fahnehjelm K, Malm G, Ygge J, Engman M-L, Maly E, Evengård B. Ophthalmological findings in children with congenital toxoplasmosis. Acta Ophtalmol In press
25. Jenum P, Stray-Pedersen B, Melby KK, 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**: 2900–6.

26. Lappalainen M, Koskela P, Hedman K, et al. Incidence of primary toxoplasma infections during pregnancy in southern Finland: a prospective cohort study. Scand J Infect Dis 1992; **24**: 97–104.
27. Guerina NG, Hsu H-W, Meissner HC, et al. the New England Regional Toxoplasma Working Group. Neonatal screening and early treatment for congenital *Toxoplasma gondii* infection. N Engl J Med 1994; **330**: 1858–63.
28. Paul M, Petersen E, Pawlowski ZS, Szczapa J. Neonatal screening for congenital toxoplasmosis in the Poznan region of Poland by analysis of *Toxoplasma gondii*-specific IgM antibodies eluted from filter paper blood spots. Pediatr Infect Dis J 2000; **19**: 30–6.
29. Naessens A, Jenum PA, Pollak A, et al. Diagnosis of congenital toxoplasmosis in the neonatal period: a multicenter evaluation. J Pediatr 1999; **135**: 714–9.
30. Wallon M, Dunn D, Slimani D, Girault V, Gay-Andrieu F, Peyron F. Diagnosis of congenital toxoplasmosis at birth: what is the value of testing for IgM and IgA? Eur J Pediatr 1999; **158**: 645–9.
31. McAuley J, Boyer KM, Patel D, et al. Early and longitudinal evaluations of treated infants and children and untreated historical patients with congenital toxoplasmosis: The Chicago collaborative treatment trial. Clin Infect Dis 1994; **18**: 38–72.
32. Guerina NG, Ware J, Burbridge J, et al. and the Massachusetts toxoplasma study group. Pediatric Research, abstract San Diego, Ca 7–11 May 1995; **37, 4**: 1735.