Incidence of *Toxoplasma gondii* Infection in 35,940 Pregnant Women in Norway and Pregnancy Outcome for Infected Women

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Received 30 December 1997/Returned for modification 24 February 1998/Accepted 8 June 1998

From 1992 to 1994 a screening program for detection of specific *Toxoplasma gondii* antibodies involving 35,940 pregnant women was conducted in Norway. For women with serological evidence of primary *T. gondii* infection, amniocentesis and antiparasitic treatment were offered. The amniotic fluid was examined for *T. gondii* by PCR and mouse inoculation to detect fetal infection. Infants of infected mothers had clinical and serological follow-up for at least 1 year to detect congenital infection. Of the women 19.9% were infected before the onset of pregnancy. Forty-seven women (0.17% among previously noninfected women) showed evidence of primary infection during pregnancy. The highest incidence was detected (i) among foreign women (0.60%), (ii) in the capital city of Oslo (0.46%), and (iii) in the first trimester (0.29%). Congenital infection was detected in 11 infants, giving a transmission rate of 23% overall, 13% in the first trimester, 29% in the second, and 50% in the third. During the 1-year follow-up period only one infant, born to an untreated mother, was found to be clinically affected (unilateral chorioretinitis and loss of vision). At the beginning of pregnancy 0.6% of the previously uninfected women were falsely identified as positive by the Platelia Toxo-IgM test, the percentage increasing to 1.3% at the end of pregnancy. Of the women infected prior to pregnancy 6.8% had persisting specific immunoglobulin M (IgM). A positive specific-IgM result had a low predictive value for identifying primary *T. gondii* infection.

Infection by the intracellular parasite *Toxoplasma gondii* is often an asymptomatic or a mild clinical disease which is not recognized (16). However, when a pregnant woman develops a primary *T. gondii* infection, the parasite may be transmitted to the fetus and cause serious damage (30). The incidence of acquired primary *T. gondii* infection during pregnancy varies greatly from country to country and ranges from less than 1 to more than 15 per 1,000 pregnancies (30). In 1978 Stray-Pedersen found an incidence of 2 per 1,000 pregnant women in Oslo, Norway (33). If this incidence is representative of the whole country and if the rate of transmission of the infection to the fetus is 50% (5), 60 infants with congenital toxoplasmosis are born each year in Norway (where there are 60,000 births annually). Most of these infections are probably not recognized, for several reasons: (i) the maternal infection may be subclinical or mild (16), (ii) the infection of the newborn infant is usually asymptomatic (1), (iii) symptoms in the infant may develop insidiously and be nonspecific (1, 30), and (iv) *T. gondii* is difficult to demonstrate as the etiologic agent when symptoms eventually emerge (2, 4).

A seroepidemiological study conducted in Norway in 1978 showed a significantly higher prevalence of *T. gondii* antibodies among blind and partially sighted children, mentally retarded children, and children with speech or behavior disorders than in healthy controls (21, 22). Hence, it is reasonable to suggest that congenital toxoplasmosis is a considerable health problem in Norway. Fetal transmission and damage may be prevented by antiparasitic treatment during pregnancy, thereby reducing the impact of this health problem (4, 15, 28, 30).

In 1992 a nationwide prospective study aimed at the prevention of congenital toxoplasmosis, including screening of pregnant women for toxoplasma-specific antibodies, was launched in Norway (35). The objectives of the project were (i) to collect information on risk factors for infection (the results have been published elsewhere [20]), (ii) to determine the prevalence of previous *T. gondii* infection among pregnant women (18), (iii) to determine the incidence of primary *T. gondii* infection in pregnant women, (iv) to determine the rate of transmission of infection to the fetus, and (v) to obtain experience shedding light on the feasibility of a serological screening program in Norway.

### MATERIALS AND METHODS

#### Enrollment

For 1 year starting in June 1992, all pregnant women in 11 of Norway’s 19 counties attending their first antenatal health care visit were invited to participate in the study. The selected counties covered all geographical and climatic regions of the country (18). All women received an information folder containing a general description of the project as well as health education and advice on specific precautions to be taken to prevent *T. gondii* infection. A total of 35,940 women were enrolled. In the study area 35,343 live births were recorded in 1993, representing 59.2% of all live births in Norway that year (32). The mean age of the women at the time of enrollment was 28.0 years (range, 14 to 48 years). Forty-four percent of the women lived in rural areas, 23% lived in Oslo, the capital city, and 33% lived in other urban areas; 7.1% of the women were classified as foreigners (18). Oslo had a significantly higher proportion of foreigners (17.1%; 95% confidence interval [CI], 16.3 to 17.9%) than other urban or rural areas (4.1%; CI, 3.9 to 4.4; $P < 0.0001$).

#### Sample collection

Serum samples, which were collected at about the 10th gestational week for compulsory syphilis testing, were examined for antibodies to *T. gondii*. Two or three times each week the local collaborating microbiological laboratories sent the collected sera to the Toxoplasma Reference Laboratory at the National Institute of Public Health, Oslo, where all analyses for toxoplasma-
specific immunoglobulin G (IgG) and IgM antibodies were completed within 1 day. (The local collaborators were as follows: Lars Vorland, Department of Microbiology, Central Hospital of Nordland, Bodø; Arne Mehl, Blood Bank, Inherited Hospital, Levanger; Torolf Moen, Department of Immunology and Blood Bank, Trondheim University Hospital, Trondheim; Reidar Hide, Department of Microbiology, Central Hospital of Møre and Romsdal, Ålesund; Olav B. Natle, Department of Microbiology, Central Hospital of Rogaland, Stavanger; Åse-Gerd Hagen, Department of Microbiology, Buskerud Central Hospital, Drammen; Einar Aandahl, Department of Microbiology, Lillehammer County Hospital, Lillehammer; and Harald Ørjasæter, Red Cross and National Hospital Blood Center, Oslo.) The results were sent directly to each woman’s physician. Reesting was requested for seronegative women at about the 22nd and 38th week of gestation. The follow-up samples were sent directly from the physician to the Toxoplasma Reference Laboratory for analysis. If a follow-up sample was not received within 4 weeks after the expected date, a reminder was sent to the woman’s physician.

If any result showed evidence of a possible primary infection, an additional serum sample was requested immediately for confirmation. If miscarriage or fetal death was reported, an additional sample was requested to clarify the likelihood of T. gondii etiology.

### Diagnosis of maternal infection

All serum samples were examined for the presence of toxoplasma-specific IgG and IgM antibodies separately (Platelia Toxo-IgG and Platelia Toxo-IgM; Sanofi Diagnostics Pasteur, Marne la Co-quette, France) (29). If toxoplasma-specific IgM was detected and/or a follow-up serum sample showed toxoplasma-specific IgG seroconversion (positive result, titer of $\geq$1:40 IU/mL), the sample was analyzed by additional specific tests: direct agglutination assay for IgG (Toxo-Screen DA IgG [positive result, titer of $\geq$40]; bioMérieux, Marcy l’Étoile, France), immunosorbent agglutination assay for IgM (Toxo-ELISA IgM [positive result, index of $\geq$29]; bioMérieux, 7, B, and the dye test (positive result, $\geq$80 IU/mL) (31).

Serological evidence of primary T. gondii infection was defined, as described by Lebec et al. (25), as (i) serocconversion during pregnancy, (ii) a significant increase of both specific IgG titer ($\geq$2-fold) and dye test titer ($\geq$4-fold), or (iii) the presence of specific IgM and a high IgG titer (dye test titer, $\geq$300 IU/mL [6, 30]).

All commercially available tests were performed according to the recommendations given by the manufacturers. The screening tests were performed with a semiautomating analyzing robot for dilution and pipetting of the sera and reagents (model RSP 8051; Tecan AG, Hombrechtikon, Switzerland), an automatic microplate washer (model 96 PW; SLT-Instruments GmbH, Salzburg, Austria), and a computer-controlled microplate reader (model 340 ATC; SLT-Instruments GmbH).

### Diagnosis of fetal infection

As soon as the primary maternal T. gondii infection was confirmed, the woman was counseled by one of the investigators (B-S. P.). An ultrasound examination of the fetus was performed, and the woman was offered amniocentesis, performed as soon as possible but no earlier than the 12th week of gestation. Amniotic fluid (10 to 20 ml) was centrifuged, and the pellet was resuspended and subsequently inoculated intraperitoneally into a mouse to detect T. gondii parasites (6, 17). Amniotic fluid (1.5 ml) was also examined by PCR to detect toxoplasma DNA (B1 gene) (14). A nested PCR test was used as previously described (17). Antipariticastic treatment including spiramycin (before the 18th week of gestation) and/or pyrimethamine, sulfonamide, and folinic acid (after the 22nd week of gestation) according to published guidelines (34) was recommended for all women.

### Diagnosis of infection in the newborn infant

At delivery, the following samples were collected, if possible: (i) cord blood for mouse inoculation, T. gondii DNA PCR analysis, and serological examination (4); (ii) amniotic fluid for mouse inoculation and T. gondii DNA PCR (17); and (iii) placental tissue for mouse inoculation (3). In addition, follow-up serum samples were routinely collected from the infants at 1, 3, 6, and 12 months of age and analyzed for toxoplasma-specific IgG, IgM, and IgA (Platelia Toxo-IgG; Sanofi Diagnostics Pasteur). The serological analyses were performed in parallel with those of the maternal serum samples collected at the time of delivery. Congenital T. gondii infection was confirmed (25) by (i) positive result for mouse inoculation test and/or PCR of amniotic fluid and/or cord blood, (ii) positive toxoplasma-specific IgM and/or IgA in a serum sample taken during the first year of life (positive antibody results in cord blood were excluded due to the possibility of contamination with maternal blood), and/or (iii) persisting toxoplasma-specific IgG or a decrease in specific-IgG titer followed by an increase during the first year of life. All infants of infected mothers were clinically examined at birth with regard to splenomegaly, hepatomegaly, purpura, and obvious neurological abnormality. The infants with confirmed infection were subsequently examined by cerebral computed tomographic scanning and by indirect ophthalmoscopy after dilation of the pupils. The hemoglobin level and the total counts of leucocytes and platelets were measured routinely after birth. Serum total and conjugated bilirubin levels were measured during the neonatal period as clinically indicated. For infected infants, general pediatric and neurologic assessments were carried out at 3 to 4, 8, and 12 months, at which times the Griffiths mental development scales were administered together with tests of hearing and vision (12). Infected infants were treated with 4-week courses of pyrimethamine, a sulfonamide, and folinic acid alternating with 4-week courses of spiramycin during the first year of follow-up (30, 34). The parents of one infected child refused postnatal treatment.
pregnancy was 0.17% (Table 2) and varied according to trimester, place of residence, age, and nationality. The incidence was higher in the first trimester than in the second and the third. The women in Oslo had an incidence (0.46%) five times that of the women in the rest of the country (0.09%; CI, 0.08–0.10). For women living in urban areas other than Oslo, the incidence was not significantly different from that for women living in rural areas (P = 0.99). Only one case of primary infection was detected in each of the three northernmost and the two inland counties included in the study.

Foreign women had a significantly higher incidence (0.60%) than Norwegian women (0.15%; CI, 0.09–0.21). However, in Oslo, which had the highest proportion of foreign pregnant women, no significant difference between these two groups was detected. No significant variation in incidence with age was detected.

(iii) Clinical symptoms and signs. Only 11 women (23%) consulted a doctor during the acute phase of the disease. However, symptoms were recorded for 30 (62%) of the women, extreme fatigue and lymphadenopathy being the most frequent clinical findings. Two women were hospitalized, one with ocular neuritis resulting in unilateral blindness and one with acute pulmonary symptoms.

(iv) Abortion. Three women with primary T. gondii infection miscarried at the end of the first trimester. They represent 6.4% of all women with primary infection and 0.3% of the women with fetal death or stillbirth examined for toxoplasma etiology. The fetal tissues were not available for parasitic examination. It could therefore not be ascertained whether these abortions were due to fetal T. gondii infection.

Two infected women, both with additional reasons, chose legal abortion. Inoculation of fetal tissues (brain, liver, and heart) into mice yielded no parasites.

Serological aspects of maternal infection. A summary of the serological results for the 47 women identified as having a primary T. gondii infection during pregnancy is presented in Table 3.

(i) Seroconversion and/or significant titer increase. Seventeen women seroconverted during pregnancy, 7 during the period between the collections of the first and the second serum samples and 10 during that between the collections of the second and the third samples. Significant titer increase, confirming the diagnosis, was observed during the first trimester for three women.

All 20 women with seroconversion or a significant titer increase were positive for toxoplasma-specific IgM during the acute phase of the disease. Seven (35.0%; CI, 14.1 to 55.9%) of these women had no detectable specific IgG in the first sample to show detectable specific IgM. IgG was detected in the follow-up samples. Specific IgM was therefore not uncommonly detected as the first serological indicator of primary infection. All 20 women were positive by the Platelia Toxo-IgM test, but two (10%) were not positive by the supplementary Toxo-ISAGA IgM test (Table 3). For 9 (45.0%; CI, 23.2 to 66.8%) of them the peak value in the dye test did not reach 300 IU/ml during pregnancy (Table 3). This finding indicated a low diagnostic sensitivity for a dye test titer of ≥300 IU/ml.

(ii) Presence of specific IgM and a dye test titer of ≥300 IU/ml. Acute infection was indicated by specific IgM and a dye test titer of ≥300 IU/ml in the first serum sample for 27 women. These samples were collected on average at week 10.2 of gestation (range, 6 to 16 weeks). Samples tested by the supplementary Toxo-ISAGA IgM test were negative for four of these women.

(iii) Positive results for toxoplasma-specific IgM. Among the 32,033 women without toxoplasma-specific IgG when tested for the first time during pregnancy, 181 were positive by the Platelia Toxo-IgM test. Only two (1.1%; CI, 0.4 to 2.6%) of them were confirmed as having a primary infection by the emergence of specific IgG by the time of collection of the follow-up sample. Thus, the false-positivity rate of the test was 0.56% (CI, 0.48 to 0.64%), and the specificity was 99.4%.

Two hundred ninety-five women whose first sample was negative (0.92%) developed a false-positive IgM reactivity against T. gondii during pregnancy, 52 became negative. Thus, the false-positivity rate of the Platelia Toxo-IgM test was 0.92% (CI, 0.70 to 1.13%). The positive predictive values for the emergence of a positive specific-IgM result, indicating primary T. gondii infection, were 5.9% (CI, 1.7 to 10.1%) for the second serum sample and 5.4% (CI, 2.1 to 8.6%) for the third sample.

Of the 3,907 women whose first sample was positive for T. gondii-specific IgG, 266 (6.8%; CI, 6.0 to 7.6%) were also positive for T. gondii-specific IgM. For 30 (11.3%; CI, 7.5 to 15.1%) of these women, a recent T. gondii infection was con-
firmed by a subsequent titer increase or by a dye titer of $\geq 300$ IU/ml.

**Fetal infection.** In total, 11 infants were infected in utero, giving an overall transmission rate of 23% (CI, 11.3 to 35.5%) (Table 4). Vertical transmission occurred for 4 of 30 (13%) women infected before the first sample was collected and for 2 of 7 (29%) women infected between the first and the second sample collections. Among these 37 women 3 (8%) were found to have infected fetuses before the treatment started, and 3 (9%) of the remaining 34 infants were documented as having congenital infection despite treatment.

Ten women were infected between the second and the third sample collections. The diagnosis was confirmed at the time of delivery, too late for treatment to prevent transmission. Of the 10 infants born to these women 5 (50%) were infected.

(i) **Prenatal diagnosis.** Amniocentesis was performed for 31 (66%) women (Table 4), and three (9.7%) of the fetuses were found to be infected with *T. gondii*. One amniotic fluid sample was positive as determined by mouse inoculation (case 5 [Table 5]), and two samples were *T. gondii* DNA positive as determined by PCR (cases 3 and 4). These fetal infections were confirmed after birth by persisting specific IgG (case 5), positive result of PCR of DNA from amniotic fluid collected at birth (case 4), or in the case where the mother refused antiparasitic treatment (case 3), positive mouse inoculation of cord blood.

(ii) **Postnatal diagnosis.** Eight infants with congenital infection were diagnosed after birth (Table 5). Three of them (cases 1, 2, and 6) had a negative prenatal diagnosis. For two of these children, the *T. gondii*-specific IgG titer increased together with the development of specific IgM and IgA in the second half-year of life (cases 2 and 6), while mouse inoculation of cord blood yielded a positive result for one infant (case 1). All three mothers were treated with spiramycin or with pyrimethamine and a sulfonamide after the amniocentesis.

Five women who were infected after the collection of the second serum sample gave birth to infected infants (Table 5). Amniocentesis was not performed for any of these women, nor were they treated, since the infection was detected around the time of delivery. All five infants had *T. gondii*-specific IgM and persistence of specific IgG in serum.

(iii) **Clinical examination and follow-up of the infants.** The mean durations of pregnancy at parturition were 39.9 weeks (range, 37 to 43 weeks) for infected women with noninfected infants and 39.8 weeks (range, 38 to 41 weeks) for women with infected infants. The mean birth weights of infected and noninfected infants were 3,497 g (range, 3,050 to 4,150 g) and 3,601 g (range, 2,240 to 4,790 g), respectively.

### TABLE 3. Results of *T. gondii*-specific antibody analyses on sera from 47 pregnant women with primary *T. gondii* infection

<table>
<thead>
<tr>
<th>Diagnostic criterion (no. of patients)</th>
<th>No. of women</th>
<th>Seroconversion</th>
<th>Significant titer increase$^a$</th>
<th>Platelia Toxo-IgM result</th>
<th>Dye test level of $\geq 300$ IU/ml</th>
<th>Toxo-ISAGA IgM result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seroconversion (17)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td></td>
<td>3</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
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<tr>
<td></td>
<td>1</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
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<td></td>
<td>6</td>
<td>+</td>
<td>-</td>
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<tr>
<td></td>
<td>2</td>
<td>+</td>
<td>-</td>
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<tr>
<td></td>
<td>1</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

| Significant titer increase (3)         |              |                |                              |                          |                                   |                      |
|                                        | 1            | -              | +                            | +                        | +                                 | +                    |
|                                        | 2            | -              | +                            | +                        | +                                 | -                    |

| Specific IgM$^+$ and dye test titer of $\geq 300$ IU/ml (27) | 23 | - | - | + | + | + |
|                                                            | 4  | - | - | + | + | - |

$^a$ Significant titer increase was detected after the first sample positive for toxoplasma-specific IgG was obtained.

### TABLE 4. Results of pre- and postnatal examination for fetal infection and outcome of pregnancy for 47 pregnant women fulfilling the criteria for primary *T. gondii* infection

<table>
<thead>
<tr>
<th>Diagnostic criterion for maternal infection$^a$</th>
<th>Total no.</th>
<th>Aminiocentesis performed</th>
<th>Prenatal diagnosis of fetal infection$^b$</th>
<th>Infected infants</th>
<th>Miscarriage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1 (total)</td>
<td>30</td>
<td>24</td>
<td>2</td>
<td>4 (13)</td>
<td>3</td>
</tr>
<tr>
<td>Specific IgM$^+$ and dye test titer of $\geq 300$ IU/ml</td>
<td>27</td>
<td>22</td>
<td>1</td>
<td>3 (11)</td>
<td>2</td>
</tr>
<tr>
<td>Significant titer increase$^c$</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>1 (33)</td>
<td>1</td>
</tr>
</tbody>
</table>

| Sample 2                                      |           |                          |                                          |                  |             |
| Sample 3                                      |           |                          |                                          |                  |             |
| Sample 2 Seroconversion                        | 7         | 7                        | 1                                        | 2 (29)           | 0           |
| Sample 3 Seroconversion                        | 10        | 0                        |                                          | 5 (50)           | 0           |

| Total                                         | 47        | 31                       | 3                                        | 11 (23)          | 3           |

$^a$ Sample 1 was collected prior to the 18th week of gestation, sample 2 was collected between the 18th and 34th weeks of gestation, and sample 3 was collected after the 34th week of gestation.

$^b$ Before antiparasitic treatment.

$^c$ Detected by analysis of the additional confirmatory sample.
Unilateral chorioretinitis, which is considered typical of toxoplasmosis, was detected in one infant at birth, and he subsequently developed strabismus and significant loss of vision in the affected eye (case 7 [Table 5]). The Griffiths development score was 95 at 1 year of age. The mother had been infected in the last trimester and had not received antiparasitic treatment during pregnancy. All the other infected infants, including the infected infant whose parents refused treatment, had normal development, with Griffiths development scores over 85, and none of them had any signs or symptoms that could be associated with congenital toxoplasmosis during the 1-year follow-up period.

DISCUSSION

Prevalence and incidence. Both the prevalence (18) and the incidence of *T. gondii* infection among pregnant women in Norway were low compared to those in other European countries (30). Although pregnant women in the Oslo area had only a slightly higher prevalence of previous *T. gondii* infection (13.2%) than other women in the study (10.2%), they had an incidence of *T. gondii* infection during pregnancy approximately five times that of women living outside the capital city. Both the prevalence and incidence seem to have remained more or less unchanged since the mid-1970s, when a similar study was performed in Oslo (18, 33). This is in contrast to the decreases in prevalence and incidence reported for some other countries (11, 16). For the country as a whole the prevalence of 10.9% and the incidence of 0.17% are markedly lower than in the neighboring countries where similar studies have recently been performed. The prevalence and incidence in Finland were 20.3 and 0.24%, respectively (23), and in Denmark a prevalence of 27.4% and an estimated incidence of 0.65% were reported (26). Further discussion of the prevalence is presented elsewhere (18).

The registered incidence of 0.17% was based on accepted diagnostic criteria (25), but the diagnoses were confirmed by seroconversion or a significant titer increase for only 20 (43%) of the 47 women with recent *T. gondii* infection. For the other 27 women the diagnoses were based on positive specific-IgM tests and a dye test titer of ≥300 IU/ml, and the infections in these cases may have occurred prior to pregnancy. The considerably higher incidence in the first trimester than in the second and third trimesters supports this possibility. Furthermore, the additional determination of *T. gondii*-specific IgG avidity in the first serum sample makes it possible to exclude latent infections more accurately (19). In Finland, this approach reduced the detected incidence to 0.28% from the 0.42% incidence obtained with the initial criteria for a recent infection (24).

However, the difference in incidence between the first trimester and the following trimesters could also partly be explained by a change in behavior after the collection of the first sample due to the information received on how to avoid infection (11).

On the other hand, a dye test titer of ≥300 IU/ml is not always present during primary infection (Table 3) (19). Therefore, cases of primary infection in which specific IgM was detected and the dye test titer was <300 IU/ml in early pregnancy may erroneously have been classified as latent infections.

Due to the problems associated with the diagnostic criteria of positivity for specific IgM and a dye test titer of ≥300 IU/ml (19), we have now included IgG avidity determination as the first supplementary test for pregnant women with toxoplasma-specific IgG and IgM. This improves the ability to exclude women infected prior to pregnancy.

Congenital toxoplasmosis. (i) Transmission of infection. The global transmission rate was 23%. The transmission rate for each trimester corresponds well to the findings of Desmonts and Couvreur (5). However, as discussed earlier, some women may have been falsely diagnosed as being infected in the first trimester. If this is the case the true denominator should be lower, which means that both the transmission rate in the first trimester and the overall transmission rate would be higher.

Despite treatment, 3 of 37 women with a negative result for prenatal examination of the amniotic fluid gave birth to infected infants. How many infants would have been infected had prenatal antibiotic treatment not been given is unknown. Assuming a transmission rate in the first and second trimesters of 25% when no treatment is given (5), it can be roughly estimated that in our study treatment may have prevented transmission of the parasite to three or four fetuses. However, if the transmission rate without treatment in the first two tri-
mesters were 16%, no transmission of infection would have been prevented. The preventive effect of prenatal treatment on vertical transmission of infection has been questioned (9).

Both Desmonts et al. (6) and Hohlfeld et al. (14) found a sensitivity of congenital toxoplasmosis detection of 64% for prenatal examination of amniotic fluid by mouse inoculation. For PCR Hohlfeld et al. found a sensitivity of 97.4% (14). In the present study only one of six (17%) women was identified as positive by mouse inoculation and two (33%) were identified as positive by PCR. The lower sensitivity may be explained by the fact that in our study the amniocentesis was performed as soon as possible after the maternal diagnosis was confirmed, but not before the 12th week of gestation, while in the other studies the amniocentesis was performed after the 18th gestational week. In a more extensive comparative study on the usefulness of PCR and mouse inoculation for the detection of congenital toxoplasmosis, members of our group have found 55% sensitivity for both methods when performed on amniotic fluid samples collected before the start of treatment (17).

Specific IgG persisting during the first year of life has been regarded as the definite criterion for congenital toxoplasma infection (6). In our study three congenitally infected infants were negative for T. gondii-specific IgG at 1 year of age. All three mothers were infected in the first trimester. The criteria for the diagnoses for these three infants were a positive PCR for T. gondii DNA in amniotic fluid, a positive mouse inoculation of cord blood, or both (Table 5). There is a possibility of false-positive PCR results if contamination of the sample occurs in the laboratory (13). But for the infant with the diagnosis based only on a positive result for PCR (case 4), PCR also yielded a positive result 3 months later for a second amniotic fluid sample, collected at birth. This indicates that detectable specific IgG does not always persist beyond 1 year of age in infants with congenital toxoplasmosis who have received antiparasitic medication. A possible modification of IgG titers by treatment is also mentioned in the classification system by Lebech et al. (25).

For one congenitally infected child (case 5 [Table 5]) the mouse inoculation of amniotic fluid yielded a positive result while the PCR was negative in the second trimester. This discrepancy may be explained by the greater volume of amniotic fluid used for mouse inoculation (10 ml) than for PCR (1.5 ml) (17) combined with a low number of parasites or by inhibitory factors affecting the PCR (13).

(ii) Clinical symptoms. The ability of the treatment to reduce damage and complications of the congenital infection could not be estimated in our study, since all but one of the infants were treated. However, among the 11 infected infants only 1 infant (9%), who was not treated prenatally, had clinical signs at birth and at 1 year of age. The effects of prenatal treatment on the frequency and severity of sequelae have been reported earlier (4, 5, 15, 28), although randomized, placebo-controlled studies of the effect of treatment on children with congenital toxoplasmosis have not been done (9) and probably cannot be done for ethical reasons.

False-positive IgM results. The Platelia Toxo-IgM test has recently been reported to give an unacceptable number of false-positive results (27). We could not confirm this. The analytic specificity of the Platelia Toxo-IgM test among pregnant women not previously infected with T. gondii was 99.4%, which must be regarded as very high. However, 6.8% of specific-IgG-seropositive women were IgM positive. It seems unlikely that the proportion of positive results for nonspecific IgM is more than 10 times higher among IgG-seropositive women than among IgG-seronegative women. The positive IgM results among IgG-seropositive women therefore probably reflect true specific-IgM results. This means that specific IgM occurs frequently among pregnant women with latent infection (19). Therefore, since the incidence of primary T. gondii infection was low in our study, the presence of specific IgM was a poor indicator of primary infection.

Compliance. The ability of an antibody screening program to detect infection occurring during pregnancy depends not only on the diagnostic sensitivities and specificities of the tests used but also on the compliance to the program. The exact participation rate in our study is not known, as the number of women who refused to participate was not recorded. However, judged by the number of live births in the study area during the period of the investigation, the participation rate was very high. However, due to lack of follow-up samples and the fact that the last samples were collected at approximately week 38 of gestation and not at delivery, on average only 34 weeks, or 85%, of each pregnancy was covered by the antibody screening program. One reason for this was probably that many women delivered before the 38th gestational week, when the third sample should have been collected. Another likely reason is that many miscarriages and legal abortions were not reported to the project administration. These pregnancies were erroneously thought to have been completed and have consequently falsely reduced the recorded compliance to the program.

REFERENCES


