

Prevention of Congenital Toxoplasmosis in Szeged, Hungary

ZSUZSANNA SZÉNÁSI,* ZSÓFIA OZSVÁR,** ERZSÉBET NAGY,* MÁRIA JESZENSZKY,** JÁNOS SZABÓ,† JÁNOS GELLÉN,† MIHÁLY VÉGH‡ AND CHRIS VERHOFSTEDÉ§

Szénási Z (Department of Clinical Microbiology, Albert Szent-Györgyi Medical University, Szeged, Hungary), Ozsvár Z, Nagy E, Jeszenszky M, Szabó J, Gellén J, Végh M and Verhofstede C. Prevention of congenital toxoplasmosis in Szeged, Hungary. *International Journal of Epidemiology* 1997; **26**: 428–435.

Background. *Toxoplasma gondii* infection of the fetus can only be discovered or prevented by the appropriate serological screening and subsequent treatment of the mother and her offspring. In Hungary, there is no obligatory toxoplasma screening for pregnant women and both the reporting and follow-up of congenital toxoplasmosis cases is limited. In 1987 we started a systematic study in the Szeged region of Hungary, in which all pregnant women were screened and appropriate treatment given to all mothers and their offspring where congenital toxoplasmosis was suspected.

Methods. All pregnant women were routinely screened within the first 16 weeks of gestation for toxoplasma antibodies by complement fixation test (CFT). Seronegative cases were retested for possible seroconversion every second month. Patients with CFT titres $\geq 1:256$ were retested for anti-P30 immunoglobulin A (IgA), IgM and IgG antibodies by ELISA and/or SDS-PAGE-Western immunoblot in order to distinguish the acute and chronic phases of the infection.

Results. Up to the end of 1994, the sera of 17 735 gravidae were screened. Ten women were found to have seroconverted during pregnancy and 78 had high initial antibody levels accompanied by anti-P30 IgA antibodies at the very first screening. These two groups together were considered as definitely (10) or possibly (78) infected with *Toxoplasma* during pregnancy and were treated with Spiramycin. All of their offspring were also treated for one month and followed-up by systematic serological and clinical screening for 2 years. No congenital toxoplasmosis was found in any of the offspring.

Conclusions. Antenatal, early diagnosis and treatment of toxoplasmosis in mothers, together with treatment and follow-up of their offspring, may considerably reduce the incidence of the disease in the offspring.

Keywords: congenital toxoplasmosis, serological screening, prevention, treatment, Hungary

Acquired maternal infection with *Toxoplasma gondii* (*T. gondii*) during pregnancy may cause congenital toxoplasmosis. Transplacentally acquired infection of the fetus may cause chorioretinitis, severe thrombocytopaenia, intracranial calcification, hepatosplenomegaly, and disturbances of head size, etc. In infected newborns, who appear normal at birth, retinal scars may develop slowly during the first 3–4 years of life, either with or without accompanying symptoms. The retinal lesion is probably the most frequent manifestation of congenital

toxoplasmosis.^{1–3} If the retinal damage occurs in the macular area, blindness may result. Each case of congenital toxoplasmosis is a tragic event for the family and a social burden to society.

Prevention of congenital toxoplasmosis is a step-wise process. The first step is reduction in the risk of contamination of women by adequate health education both before and during pregnancy. The second step comes after maternal contamination: the reduction and prevention of fetal disease by the early diagnosis and treatment of the toxoplasma infection during pregnancy. The third step involves the early treatment of the newborn and special follow-up of the child at least for 2 years. However, it is not easy to reduce the risk of contamination of pregnant women since this would demand a change in their lifestyle in order to avoid the multiple means of toxoplasma transmission, e.g. via contact with infected cat faeces, or eating insufficiently washed or unwashed raw vegetables and fruits (such as strawberries, lettuce, etc.) infected with cat faeces, or

* Department of Clinical Microbiology, Albert Szent-Györgyi Medical University, PO Box 482, H-6701 Szeged, Hungary.

** Department of Infectious Diseases, Municipal Hospital of Szeged, Szeged, Hungary.

† Department of Obstetrics and Gynaecology, Albert Szent-Györgyi Medical University, Szeged, Hungary.

‡ Department of Ophthalmology, Albert Szent-Györgyi Medical University, Szeged, Hungary.

§ Department of Bacteriology, Virology and Immunology, University Hospital, Ghent, Belgium.

the consumption of raw or undercooked meat containing tissue cysts.^{1,3-5} The walls of these cysts are quite resistant to gastric juice and thus the parasites are protected from gastric degradation.^{1,6} Information about preventive measures should be given to all women of childbearing age before pregnancy in order to prevent acute infections in early pregnancy, which is considered the most dangerous period for the fetus. Toxoplasmosis is generally asymptomatic in immunocompetent patients, therefore, it is not easily detected during pregnancy.⁷ Early identification of the infection followed by treatment is, however, the only practical way to reduce the incidence of congenital toxoplasmosis.

With this study, we have tried to evaluate the effect of an intensive programme for the prevention of congenital toxoplasmosis in Szeged (Hungary). The programme consisted of the prevention of infection of the mother by education about risk factors, and the prevention of infection of the child by early diagnosis and treatment of the infection.

SUBJECTS AND METHODS

Subjects

All pregnant women (17 735) in the city of Szeged between 1987 and 1994 were routinely screened for toxoplasma antibody. Written information was given to the women which described the symptoms and consequences of toxoplasma infection, and the aims of the screening using blood sampling. The information was provided in such a way that it would be easy for the women to understand but would not induce unnecessary anxiety. Since toxoplasma screening is not obligatory in Hungary, blood sampling was carried out only after the informed consent of the pregnant women and the approval of the Ethics Committee of the University.

Antenatal Screening and Maternal Treatment

The most important feature of antenatal screening is the date of the first blood sample. The sooner this is done, the better, and the pregnant women were therefore tested when they were first seen at antenatal clinics, between the 8th and the 16th weeks. At this time, a compulsory blood sample is taken for syphilis examination, thus, the same blood sample was also used for the determination of toxoplasma antibody. Thus, the first blood sample involved no extra cost and the pregnant women had no extra stress with regard to toxoplasma screening. In women with complement fixation test (CFT) titres of 1:16–1:128 at this time further tests were omitted on the assumption that they had been infected before the pregnancy.³ This greatly reduced the cost of the serological surveillance. If the CFT titre was $\geq 1:256$ at

the first test, the specimen was further examined either by SDS-PAGE-Western immunoblot⁸⁻¹⁶ or by the determination of anti-P30 IgA and IgM^{9,17-19} in order to distinguish between the 'acute' and 'chronic' phases of the infection. (P30 is a major surface protein of *T. gondii* expressed only by tachyzoites.) Patients with high CFT titres but no anti-P30 IgA antibody titres were considered as having 'chronic' infection, thus, with no risk of recent toxoplasmosis, therefore, further testing was omitted. In contrast, patients with high CFT titres *plus* anti-P30 IgA antibody titres were considered to have recently acquired Toxoplasma. A minority of these infections may have been acquired during the pregnancy but the time cannot be determined exactly by serological methods. Seronegative cases were retested every second month until the end of pregnancy to monitor any seroconversion. When recently acquired toxoplasma infection was serodiagnosed (e.g. we discovered seroconversion or rising CFT titres, or continuous high CFT titres with the presence of anti-P30 IgA antibody), the mother was immediately treated with 3 g of Spiramycin per day until one month before the expected delivery. To confirm fetal infection, a fetal blood sample was taken from the umbilical vein. This was also done in three of the ten seroconverted mothers with the consent of the mother.²⁰ No consent was given by the other seven patients. The fetal blood was used for Western blot analysis and/or anti-P30 IgA, IgM determinations by ELISA, as well as for inoculation into mice and polymerase chain reaction (PCR) amplification. The same tests were also performed on amniotic fluid obtained by amniocentesis at the time of the fetal blood sampling.²¹ The amniotic fluid from two patients was also used for PCR-amplification of part of the P30 gene of the parasite.²² Ultrasound examination was also performed monthly from the time of fetal blood sampling up to the end of pregnancy, paying special attention to the size of the lateral ventricles, signs of intracranial calcification, the thickness of the placenta, and fetal hepatomegaly.

Neonatal Surveillance and Neonatal Treatment

At birth, cord blood was taken for anti-P30 IgA and IgM determination by ELISA and/or Western immunoblot in all cases where the mothers displayed serological evidence of acute infection. Two weeks later, a blood sample was also taken from the baby for the same examinations.⁸⁻¹¹ Serological examinations were repeated monthly up to 6 months of age, and then every 6 months up to 2 years of age. At these points in time, ophthalmoscopy, ultrasound examination and skull radiography were also performed. From birth, the newborns of the infected mothers were treated with 500 mg per 5 kg of Spiramycin per day for one month.

TABLE 1 Immune status to toxoplasmosis during pregnancy over an 8-year period

Year	No. of gravidae (Groups A+B)	Group A Seronegative subjects		Group B Seropositive subjects		Group C Seroconverted subjects		Group D HIAL with anti- P30 IgA +ive ^c		Groups C+D (Treated with Spiramycin)	
		No.	(%)	No.	(%)	No.	(%) ^b	No.	(%) ^d	No.	(%) ^e
1987 ^a	1758	718	(40.8)	1040	(59.2)	1	(0.14)	7	(0.67)	8	(0.45)
1988	2199	805	(36.6)	1394	(63.4)	1	(0.12)	4	(0.29)	5	(0.22)
1989	2270	890	(39.2)	1380	(60.8)	4	(0.45)	5	(0.36)	9	(0.40)
1990	2218	980	(44.2)	1238	(55.8)	2	(0.20)	3	(0.24)	5	(0.23)
1991	2491	668	(26.8)	1823	(73.2)	0	(0)	13	(0.71)	13	(0.52)
1992	2273	670	(29.5)	1603	(70.5)	1	(0.15)	12	(0.74)	13	(0.57)
1993	2299	823	(35.8)	1476	(64.2)	0	(0)	14	(0.95)	14	(0.61)
1994	2227	691	(31.0)	1536	(69.0)	1	(0.16)	20	(1.43)	21	(0.94)
Total	17 735	6245	(35.2)	11 490	(64.8)	10	(0.16)	78	(0.68)	88	(0.50)

^a Study started on 9 February 1987.

^b Percentage of seronegative women seroconverting during pregnancy.

^c HIAL with anti-P30 IgA +ive = high initial antibody levels accompanied by anti-P30 IgA antibodies.

^d Percentage of seropositive patients with HIAL and anti-P30 IgA.

^e Percentage of gravidae with seroconversion and HIAL with anti-P30 IgA.

Serological Methods

All sera were tested by a sensitive CFT (the dilution of the complement was usually 1:90, and the dilution of the haemolysin was 1:300), using a toxoplasma antigen containing both the mannan and the somatic components of *T. gondii* (SEVAC, Prague, Bohemia).¹⁰ Seronegative cases were retested by CFT every second month to monitor seroconversion. If the CFT titre was $\geq 1:256$ at the first testing, the specimen was further examined by ELISA IgG (Organon Teknika, B V Boxtel, Holland, or Sanofi Diagnostic Pasteur, Paris, France) and either by the technique of SDS-PAGE and Western immunoblot with anti-human IgG-conjugate (REANAL, Budapest, Hungary),^{8,11} or by anti-P30 IgA and IgM immunocapture ELISA kits (Sanofi Diagnostic Pasteur, Paris, France) using the P30 protein as antigen.⁹ The PCR amplification of part of the P30 gene of *T. gondii*,²² Western blot analysis^{8,11} and/or anti-P30 IgA, IgM determinations,¹⁷ and inoculation into mice were carried out also from fetal blood and amniotic fluid.

The sensitivity, specificity, and positive predictive value (PPV) of the CFT were calculated by comparing with the ELISA IgG (Sanofi Diagnostics Pasteur, Paris, France) as a reference test. (According to the Sanofi Diagnostics Pasteur's brochure, the sensitivity and the specificity of the ELISA IgG are 100.0% and 99.6%, respectively, as compared to the indirect immunofluorescence.) Altogether, 192 random samples were examined. Sensitivity was found to be 97.1%, specificity: 86.2%, and PPV: 89.5%.

RESULTS

Table 1 summarizes the data on the immune status to *T. gondii* infection of 17 735 pregnant women according to the year in which they were screened. The prevalence of the presence of *T. gondii* antibody varied between 55.8% in 1990 and 73.2% in 1991. In all 6245 women (35.2% of those screened during the study) were seronegative at the first test. Altogether, ten patients were found in the seroconverted group (Group C), ranging from zero in 1991 and 1993 to four (0.45%) in 1989. The yearly distribution of Group D (high initial antibody levels [HIAL] with anti-P30 IgA) varied from three (0.24%) in 1990 to 20 (1.43%) in 1994. The patients in Groups C+D varied from five in 1988 and 1990 (0.22% and 0.23%, respectively) to 21 cases (0.94%) in 1994. Thus, a total of 88 patients, (Groups C+D) were found with serological evidence of acute toxoplasmosis. All these patients were treated immediately and continuously, as mentioned earlier. The 88 babies born to these mothers were treated for one month and followed up for 2 years, as described earlier. No case of congenital toxoplasmosis was discovered during this study. Rigorous surveillance during the whole period of the study could not discover any side effects of the Spiramycin treatment either in the mothers or in the infants.

The IgG immunoblot pattern and the anti-P30 IgA antibody levels were determined in 27 pregnant women (Groups C+D) from 1987 to 1990. The appearance of the 30 kD early band on immunoblot was seen together with the appearance of anti-P30 IgA antibody. These

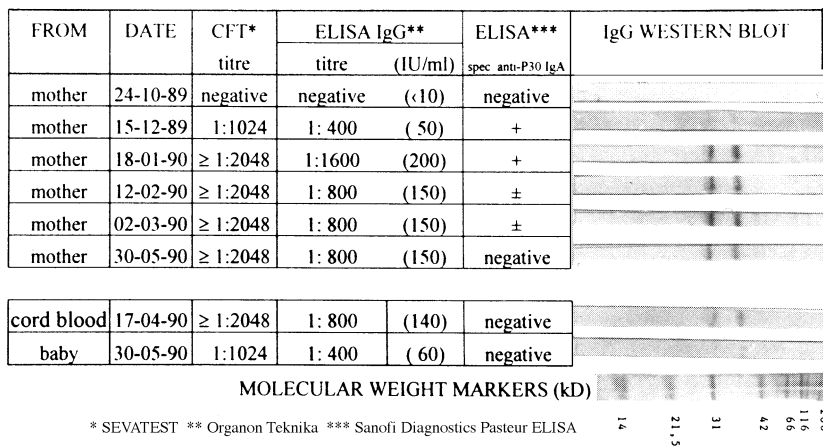


FIGURE 1 IgG immunoblot pattern and antibody titres of a pregnant woman with seroconversion, and of her baby

were found to be early indicators of an acute infection. A fading of the 30 kD band on the immunoblot (in parallel with the decrease in the anti-P30 IgA antibody level) seems to indicate the transition of the acute phase to the chronic phase. The transition was observed 3–8 months after seroconversion.

Figure 1 illustrates the IgG immunoblot pattern, the CFT titres and the ELISA IgG and IgA results of a pregnant woman with asymptomatic toxoplasmosis, together with the pattern of her newborn. The 30 kD and the 35 kD molecular weight early bands appeared (in parallel with the anti-P30 IgA positivity) in the second serum sample, taken 2 months after the last seronegative sample. The anti-P30 IgA decreased below the cutoff level within 4 months. At that time, the CFT and ELISA IgG titres were still high. The immunoblot patterns of the cord blood and the blood of the newborn were identical. The intensity of the bands declined during the first month of the baby's life, indicating that the baby's pattern originates from the passively transferred maternal IgG antibodies. The baby had an elevated CFT titre, but not detectable IgA antibodies or clinical symptoms.

Figure 2 shows the results of a patient from Group D. She had constantly high levels of CFT and ELISA IgG, with the presence of anti-P30 IgM and IgA. The anti-P30 IgA titre decreased within 8 months whereas the IgM persisted for more than one year.

DISCUSSION

Since acquired toxoplasmosis is generally asymptomatic among immunocompetent patients, it is rarely

detected during pregnancy. Thus, their offspring can escape the possibility of infection only through the early identification of the infection in their mother by prospective screening, and by subsequent appropriate treatment.^{7,18}

The finding that IgM antibody, which is a marker of a recent infection, can persist occasionally long after the onset of toxoplasmosis, complicates the serodiagnosis of a recent infection.^{17,18} IgG may also persist in high titres for more than one year after seroconversion. Thus, the persistence of high titres of IgM and IgG, do not allow an estimate of the onset of the infection, which is especially important in pregnant women where only acute infections can be transmitted to the fetus.

In congenital toxoplasmosis, the specific anti-toxoplasma IgM antibody is absent in one-third of the babies.²³ Further, an elevated anti-toxoplasma IgG antibody titre in the newborn may be of either maternal or fetal origin, i.e. it may reflect either an active congenital toxoplasmosis or simply the passively transferred antibodies of a maternal toxoplasmosis.

The detection of specific IgA against *T. gondii* P30 has recently been used for the differential diagnosis of acute and congenital toxoplasmosis.^{17,24} This 30 kD protein is the major surface protein of the multiplicative form of toxoplasma. It comes into contact with the immune system very early after infection, and thus it is a favoured target of the acute humoral immune response. In our study, the fast developing anti-P30 IgA titres usually decreased more rapidly than the IgM titres. Thus, we could confirm that anti-P30 IgA ELISA is a useful, easy-to-handle method for the determination of a recently acquired toxoplasmosis (Figure 2).

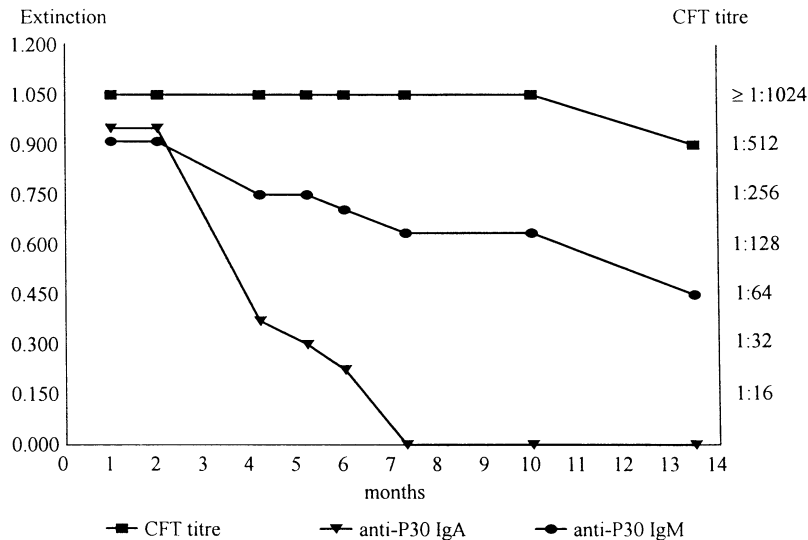


FIGURE 2 Fresh infection of a patient discovered with high levels of IgA, IgM and high complement fixation test (CFT) titre

Furthermore, no IgA antibody was found in any of the newborns born to infected mothers. These babies proved to be uninfected during the follow-up.

Beside the IgA determination, we evaluated the technique of SDS-PAGE-Western immunoblot. The SDS-PAGE-Western immunoblot reveals a characteristic pattern evolved by the binding of antibodies to distinct toxoplasma antigenic components. The changes in the immunoblot pattern are considered to indicate the stage of infection.⁸⁻¹² Using immunoblot we found (Figure 1) that the 30 kD and the 35 kD molecular weight antigen components elicited an IgG response early after the onset of the infection. The intensities of these bands decreased earlier than the CFT and/or ELISA IgG titres, i.e. the fading of the early bands seems to indicate the transition of the infection to the chronic stage earlier than the decrease in the CFT and the IgG ELISA titres. Thus, determination of the immunoblot pattern is a useful diagnostic tool in the serodiagnosis of the stages of toxoplasmosis of the mother in the event of persistent high titres of IgM and/or IgG. In the baby, the pattern of the immunoblot indicates that the infant did or did not acquire toxoplasmosis *in utero*. Since 1991, the regular IgG immunoblot determination has been omitted; first, because of the parallelism observed between the results of this test and the results of IgA determination, second, because the ELISA method of anti-P30 IgA determination is cheaper, easier to perform and less time-consuming than the SDS-PAGE-Western blot technique and the interpretation can be performed by the technical

staff. It should be stressed, however, that the IgG immunoblot determination may be extremely useful in analysing some disputed cases.

An important part of any 'case finding' screening study is the ability of the test to classify as positive people with the disease (sensitivity) and to class as negative those without the disease (specificity).²⁵ According to Wilson & Jungner, a test should be, first, highly sensitive; second, as simple as possible; third, cause minimal disturbance to the subject.²⁵ We feel that our screening test fulfils these criteria.

Table 1 shows data on the 17 735 pregnant women we screened between 1987 and 1994. The prevalence of antibody to *T. gondii* was determined each year from 1987 to 1994 and varied between 55.8% and 73.2%. This value is definitely higher than those found earlier in Hungary in some less detailed studies,²⁶⁻²⁸ and is somewhat lower than those found in the neighbouring Yugoslavia,²⁹ while, in Austria, Aspöck found a decrease from 50% in 1975 to 36% in 1994.³⁰ Of the 6245 seronegative subjects (35.2% of all screened patients), ten patients seroconverted during the screening period, and thus were in danger of passing toxoplasma infection to their offspring (Group C). Published data^{13,31} indicate that 10-30% of infants of uninfected and untreated infected mothers will have impairment. However, the actual rate of infection of the offspring depends on several factors: virulence of toxoplasma strains, immunological status of the pregnant women, and, in particular the age of the fetus at the time of exposure.²⁹ Thus, the

frequency of fetal infection is considered approximately 15% in the first trimester, 25% in the second, and 60% during the third trimester.³² A 90% infection rate may occur in the last weeks before delivery.³³ The severity of the fetal damage shows, however, the opposite tendency: a decrease from the first trimester to the third trimester.³⁴ Aspöck assumes a 50% risk of toxoplasma infection of the infected mothers' fetuses.³⁰ Thus, it is not unreasonable to suppose that at least five fetuses might have been infected with toxoplasma had not therapy started immediately after the discovery of seroconversion.

In addition, Group D (high initial antibody levels accompanied with anti-P30 IgA antibodies) may also be at risk of transmission of toxoplasmosis to their fetuses, considering that the 78 pregnant women in this group had definitely had a recent, acute toxoplasma infection. Some of these might have occurred after the time of conception in that, the first blood sample in our study was done between the 8th and 16th weeks of pregnancy, the majority between the 12th and 16th weeks. It is not unreasonable to suppose that in approximately one-third of this 'acute' group (i.e. 13–17 women), the toxoplasma infection might have occurred after conception. Using a 50% fetal transmission rate, 7–9 offspring could have been infected without the screening and treatment programme. Conservatively, we might have expected 12–14 infected offspring from Group C+D. In our study, however, not a single case of congenital toxoplasmosis was found among the 88 offspring of mothers of Group C+D who were screened antenatally and treated appropriately. In concordance with the results of Stray-Pedersen,³³ no adverse effects of Spiramycin treatment were observed during the 8 years of the study.

No such screening (and hence, no such treatment) has been performed in other regions of Hungary. The comparison of our impressive results (i.e. no neonatal toxoplasmosis at all) with the whole of Hungary is very difficult. The number of live births for Hungary from 1987 to 1994 was 981 083;³⁵ the annual mean was 122 635 with 116 000 and 127 207 as extremes in 1994 and 1991, respectively. However, the annually reported number of toxoplasma cases in the entire country during the same period was much more variable, 15 in 1987, 16 in 1988, five in 1989, three in 1990, two in 1991, two in 1992, one in 1993, and two in 1994; 46 in total. (Data obtained from the 'Béla Johan' National Institute of Public Health, Budapest.) The same data relating to the 8 years (1979–1986) preceding our studies are as follows: the number of live births was 1 078 957 altogether,³⁵ and the annual mean was 134 869. The reported number of congenital toxoplasma cases during the same period was exactly 100 with three (1979) and

31 (1986) as extremes. Does it mean a drastic decrease in the incidence of neonatal toxoplasmosis in recent years? Or, does it mean a drastic decrease in the (obligatory) reporting of neonatal toxoplasma cases to the 'Béla Johan' National Institute of Public Health? Or, are there other unknown factors causing a rapid decrease of the reported cases of the neonatal toxoplasmosis? Ljungström supposes a significant underestimate of congenital toxoplasmosis in Sweden because the infected neonate may be without symptoms and still be at risk of developing sequelae such as chorioretinitis.³⁶ Anyway, it must be stressed that our report is not able to answer that question definitely, nor was its purpose to unravel the reasons.

An unavoidably important aspect in all screening programmes is the evaluation of the cost-benefit estimates related to other methods, e.g. clinical treatment. Such calculations were not commonly applied previously in Hungary. Even now, many of the costs are continuously changing, or difficult to estimate due to a lack of proper information and uncertainties in health policy. Nevertheless we have tried to give some calculations, but these may not be definitive enough to make a final decision on the cost-effectiveness of screening *versus* other methods in the case of congenital toxoplasmosis.

The most important costs of the screening programme derive from the serological tests. In our calculation, the yearly cost of CFT is approximately 450 000 Hungarian Forints (HuFt). (In May 1996, 1 HuFt = 0.006587 US\$.) Confirmatory tests (ELISA IgA/IgM/IgG): 571 000 HuFt. Thus, the serological tests cost around 1 021 000 HuFt. Gynaecological screening (together with Spiramycin treatment) for the suspected cases (Group C+D): 153 000 HuFt approximately. Costs of ophthalmological screening: 40 000 HuFt per year. Neurological tests: 65 000 HuFt. Infection screening 17 000 HuFt approximately. The total costs of the screening programme have been 1 296 000 HuFt per year, i.e. 10 368 000 HuFt for the 8-year period of our study.

We estimated that about 12–14 infected offspring would have occurred in the Szeged region during our study without the screening programme. Thus, the prevention of each potential congenital toxoplasmosis has cost 740 000–864 000 HuFt. According to the data of one of us (ZO) the government's Department of Health allows 500 000 HuFt spending per year for each child with toxoplasmosis requiring institutional care. This is far from being enough: the actual costs of acceptable care, however, are at least 850 000 HuFt. Additional costs of home care, travel costs, working time lost by parents and patients, etc. are not included. In conclusion, the costs of the yearly screening programme are in

the same range as the costs of the institutional care of one case of congenital toxoplasmosis. Lappalainen states that severely ill children require permanent institutional care for the whole of their lives, i.e. 40 years.³⁷

To conclude, we believe that the introduction of a nationwide toxoplasma screening programme would result in a decrease in neonatal toxoplasma cases like that experienced in the city of Szeged in other regions of Hungary. A programme using the CFT as a first line screen, and anti-P30 toxoplasma IgM and IgA determination with occasional Western immunoblot, as additional tests to discriminate between acute and chronic infections, seems to be efficient. Further studies need to be performed on the more accurate determination of the risk of materno-fetal toxoplasmosis as well as on the exact costs of home care and hospitalization, or permanent institutional care in order to make definitive cost-benefit analysis of screening versus other alternatives.

ACKNOWLEDGEMENTS

This study was supported in part by research grants from the Ministry of Welfare (ETT No. T-10, 555/93) and the Foundation for Study of Infection in the Population of Szeged and its Surroundings. We are grateful to Sanofi Diagnostics Pasteur^R for supplying some diagnostic materials.

REFERENCES

- Feldman H A. Epidemiology of toxoplasma infections. *Epidemiol Rev* 1982; **4**: 204–13.
- Hall S M. Congenital toxoplasmosis. *Br Med J* 1992; **305**: 291–97.
- Henderson J B, Beattie C P, Hale E G, Wright T. The evaluation of new services: Possibilities for preventing congenital toxoplasmosis. *Int J Epidemiol* 1984; **13**: 65–72.
- Foulon W, Naessens A, Lauwers S, De Meuter F, Amy J-J. Impact of primary prevention on the incidence of toxoplasmosis during pregnancy. *Obstet Gynecol* 1988; **72**: 363–66.
- Jeannel D, Niel G, Costagliola D, Danis M, Traore B M, Gentilini M. Epidemiology of toxoplasmosis among pregnant women in the Paris area. *Int J Epidemiol* 1988; **17**: 595–602.
- Jacobs L, Remington J S, Melton M L. A survey of meat samples from swine, cattle and sheep for the presence of encysted toxoplasma. *J Parasitol* 1960; **46**: 11–14.
- Joss A W L, Skinner L J, Chatterton J M W, Chisholm S M, Williams H D, Ho-Yen D O. Simultaneous serological screening for congenital cytomegalovirus and toxoplasma infection. *Public Health* 1988; **102**: 409–17.
- Szénási Z, Praznovszky T, Kiss I. IgG immunoblot pattern to *Toxoplasma gondii* as a marker of the stage of infection. *Bull Soc Franc Parasitol* 1990; **8**: 980.
- Szénási Z, Ozsvár Z, Jeszenszky M, Kálmán M, Zimányi M. Diagnostic value of anti-P30 specific IgA antibodies in the determination of the stage of toxoplasmosis. *Acta Microbiol Immunol Hung* 1994; **41**: 335.
- Szénási Z, Ozsvár Z, Nagy E. Latex agglutinációs teszt használata toxoplasma ellenanyag szűrésére. *Klin Kísérlet Lab Med* (Latex agglutination test for screening antibodies to *Toxoplasma gondii*. *Clin Exp Lab Med*) 1995; **22**: 13–18.
- Verhofstede C, Van Gelder P, Rabaey M. The infection-stage-related IgG response to *Toxoplasma gondii* studied by immunoblotting. *Parasitol Res* 1988; **74**: 516–20.
- Kasper L H. Identification of stage specific antigens of *T. gondii*. *Infect Immun* 1989; **57**: 668–72.
- Sharma S D, Mullenax J, Araujo F G, Erlich H A, Remington J S. Western blot analysis of the antigens of *T. gondii* recognized by human IgM and IgG antibodies. *J Immunol* 1983; **131**: 977–83.
- Rodriguez C, Afchain D, Capron A, Dissous C, Santoro F. Major surface protein of *T. gondii* (P30) contains an immunodominant region with repetitive epitopes. *Eur J Immunol* 1985; **15**: 747–49.
- Gross U, Roos T, Appoldt D, Heesemann J. Improved serological diagnosis of *Toxoplasma gondii* infection by detection of immunoglobulin A (IgA) and IgM antibodies against P30 by using the immunoblot technique. *J Clin Microbiol* 1992; **30**: 1436–41.
- Daffos F, Forestier F, Capella-Pavlovsky M *et al*. Pre-natal management of 746 pregnancies at risk for toxoplasmosis. *N Engl J Med* 1988; **318**: 271–75.
- Decoster A, Darcy F, Caron A *et al*. Anti-P30 IgA antibodies as prenatal markers of congenital toxoplasma infection. *Clin Exp Immunol* 1992; **87**: 310–15.
- Derouin F, Rabian-Herzog C, Sulahian A. Longitudinal study of the specific humoral and cellular response to *T. gondii* in a patient with acquired toxoplasmosis. *J Clin Lab Immunol* 1989; **30**: 97–102.
- Santoro F, Afchain D, Pierce R, Cesborn J Y, Ovlaque G, Capron A. Serodiagnosis of toxoplasma infection using a purified parasite protein (P30). *Clin Exp Immunol* 1985; **62**: 262–69.
- Daffos F, Capella-Pavlovsky M, Forestier F. Fetal blood sampling during pregnancy with use of a needle guided by ultrasound: a study of 606 consecutive cases. *Am J Obstet Gynecol* 1985; **153**: 655–60.
- Derouin F, Thulliez P, Candolfi E, Daffos F, Forestier F. Early prenatal diagnosis of congenital toxoplasmosis using amniotic fluid samples and tissue culture. *Eur J Clin Microbiol Infect Dis* 1988; **7**: 423–25.
- Verhofstede C, Reniers S, Colebunders R, Van Wanzele F, Plum J. Polymerase chain reaction in the diagnosis of *Toxoplasma* encephalitis. *AIDS* 1993; **7**: 1539–41.
- Naot Y, Desmots G, Remington J S. IgM enzyme-linked immunosorbent assay test for the diagnosis of congenital toxoplasma infection. *J Pediatrics* 1981; **98**: 32–36.
- Decoster A, Caron A, Darcy F, Capron A, Niel G. Platelia Toxo IgA, a new kit for early diagnosis of congenital toxoplasmosis by detection of anti-P30 immunoglobulin A antibodies. *J Clin Microbiol* 1991; **29**: 2291–95.
- Wilson J M G, Jungner G. *Public Health Papers No. 34: Principles and Practice of Screening for Disease*. Geneva: World Health Organization, 1968.
- Csóka R, Dán P. Toxoplasma antitestek előfordulása normál populációban. (Toxoplasma antibodies in the normal population.) *Orv Hetilap* 1971; **112**: 258–60.
- Korányi G, Krausz J, Remenár É. Kongenitális toxoplasma fertőzés súlyos esetei. (Grave cases of congenital toxoplasmosis.) *Gyermekgyógyászat* 1979; **30**: 243–49.

- ²⁸ Zsibrita Á, Kárai L. Egy vidéki város és környéke toxoplasmosis fertőzöttségének vizsgálata gravidákon. (Screening for toxoplasma infection in a provincial town and its vicinity in pregnant population.) *Magy Nőorv Lapja* 1983; **46**: 89–91.
- ²⁹ Djurkovic-Djakovic O. Toxoplasma infection and pathological outcome of pregnancy. *Gynecol Obstet Invest* 1995; **40**: 36–41.
- ³⁰ Aspöck H. Protozoen als Erreger von Krankheiten des Menschen: Übersicht und aktuelle Probleme in Mitteleuropa. *Kataloge des OÖ Landesmuseums N F* 1994; **71**: 219–66.
- ³¹ Jeannel D, Costagliola D, Niel G, Hubert B, Danis M. What is known about the prevention of congenital toxoplasmosis. *Lancet* 1990; **336**: 359–62.
- ³² Desmots G, Couvreur J. Toxoplasmose congénitale. Étude prospective de l'issue de la grossesse de 542 femmes atteintes de toxoplasmose acquise en course de gestation. *Sem Hôp Paris* 1986; **62**: 1418–22.
- ³³ Stray-Pedersen B. Treatment of toxoplasmosis in the pregnant mother and newborn child. *Scand J Infect Dis* 1992; **84 (Suppl.)**: 23–31.
- ³⁴ Couvreur J, Desmots G, Tourner G, Szustérkac M. Étude d'une série homogène de 210 cas de toxoplasmose congénitale chez des nourrissons âgés de 0 à 11 mois et dépistés de façon prospective. *Ann Pédiatr (Paris)* 1984; **31**: 815–19.
- ³⁵ Demográfiai Évkönyv. Budapest, Központi Statisztikai Hivatal, 1992.
- ³⁶ Ljungström 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.
- ³⁷ Lappalainen M, Sintonen H, Koskiniemi M *et al.* Cost-benefit analysis of screening for toxoplasmosis during pregnancy. *Scand J Infect Dis* 1995; **27**: 265–72.

(Revised version received May 1996)