

CONGENITAL TOXOPLASMOSIS IN PREMATURE TWINS*

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Abstract. In the course of the study "Toxoplasmosis and Prematurity" 330 blood samples from twins were examined. Our findings in a series of 21 premature twins (maternal sera were also examined) are reported in this paper. *Toxoplasma* antibodies were detected by the Sabin—Feldman test and specific IgM antibodies by the Remington test. The classical form of congenital toxoplasmosis was present in five pairs of twins, while toxoplasmosis was subclinical at birth in both twins of three pairs. The pattern of disease varied very much in seven pairs of twins. In one twin of two pairs signs of disease were present, while his cotwin appeared unaffected but with strongly positive result of SFT. The most interesting observation, however, is that in three pairs, one twin was infected and had evident congenital toxoplasmosis, while his cotwin was not, as proven by the disappearance of the *Toxoplasma* antibodies. This finding undoubtedly indicates the importance of whether the placenta is intact or not for the transmission of the infection.

A transplacental infection of the fetus with *Toxoplasma gondii* occurs only if a woman becomes infected during pregnancy (Desmonts 1982). However, not all women who contract the disease while pregnant transmit the infection to the fetus. The fact that the permeability of the placenta increases as the pregnancy proceeds (Campinchi et al. 1977), reaching its maximum in the last term, accounts for the highest frequency of infection in that period, which results most often in premature birth.

According to our data (Šulović et al. 1971, Šibalić et al. 1975), the prevalence of *Toxoplasma* antibodies in women of reproductive age amounts to about 50 %. This means that there still remains a considerable number of women which can be infected during pregnancy, and who can transmit the infection transplacentally to the fetus.

In the course of the study "Toxoplasmosis as a Cause of Prematurity" (Šibalić et al. 1979) we dealt with a significant number of neonates born as twins. A pair of twins in which one infant was infected while the other one was not aroused our interest in studying a series of premature infants born as twins.

MATERIAL AND METHODS

Among 1270 infants examined, there were 330 neonates born as twins. Of that material, we are now reporting on 21 pairs of twins for whom we could obtain complete data, including material for laboratory examinations and genetical and clinical findings.

To demonstrate *Toxoplasma* antibody, two serological tests were used: the Sabin—Feldman (SFT) dye test (1948) as modified by Desmonts (1955) to the lysis test (LT) and the indirect immuno-fluorescent (IFA) test (Ambróise—Thomas 1969); the presence of the IgM antibodies was examined by the Remington (Remington et al. 1968, Remington and Klein 1976) (IgM—IFA) test. However, the diagnosis relied most heavily on the results of the SFT. The serum was diluted 1 : 10; 1 : 100; 1 : 1 000 and higher, if necessary, for the SFT, while for the IgM—IFA test the dilutions were 1 : 2; 1 : 10; 1 : 50; 1 : 100 and higher. The titre 1 : 2 was considered positive if it was followed by an increase in the next serum sample, and negative if it was not.

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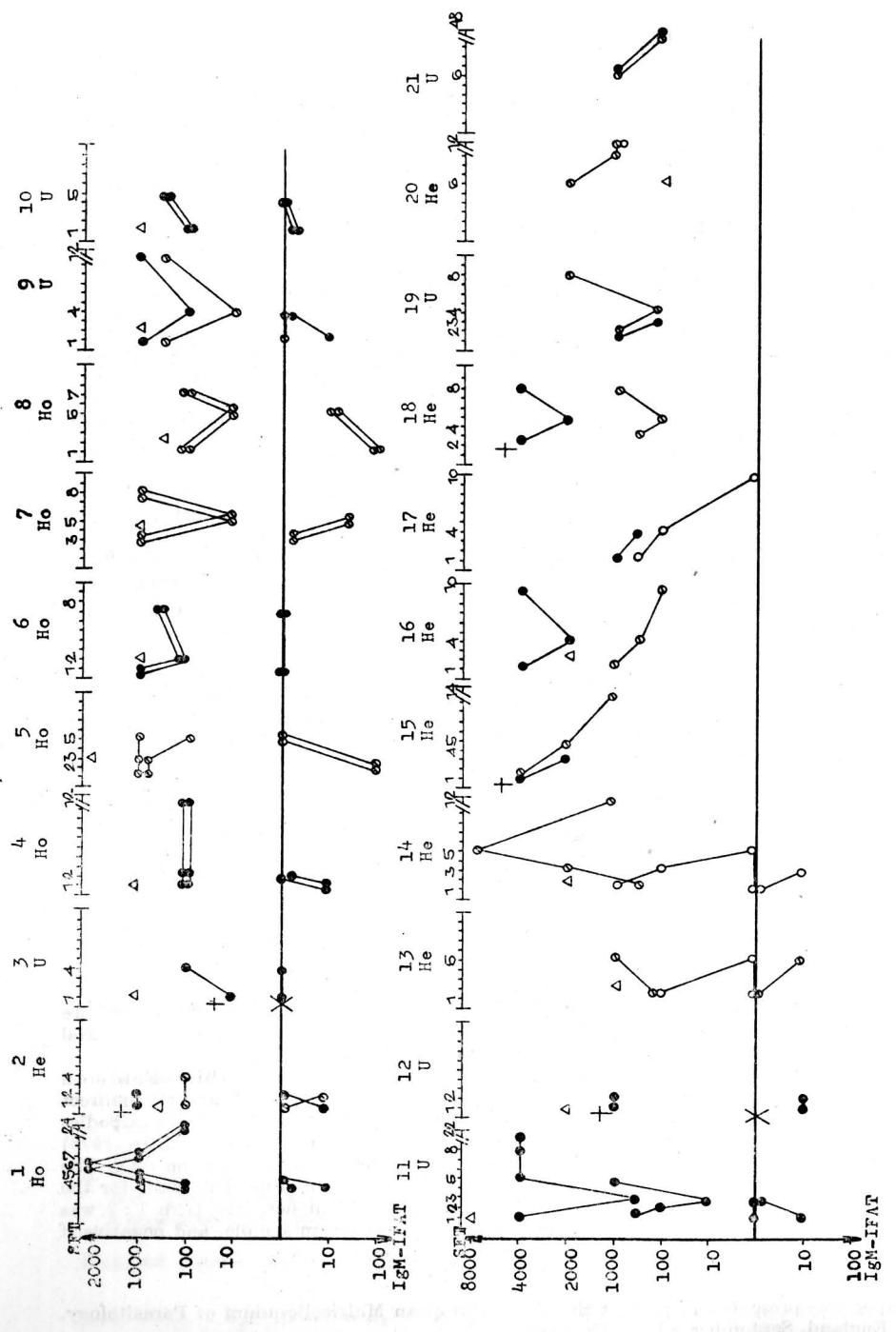


Fig. 1. Serological follow-up (SFT and IgM-IFAT) in 21 pairs of premature twins with congenital toxoplasmosis and their mothers.
 1-21 — pair number, He — homozygous pair, Ho — heterozygous pair, U — karyotype undetermined, 1,2 etc. — age in months at serological testing, o — unaffected infant, e — without clinical manifestations at birth, • — clinical manifestations present at birth, + — exstis letalis later, ▲ — infant macerated or stillborn.

Besides these tests, an attempt was made to isolate the parasite by inoculation of our patients' peripheral blood into mice. If parasites were present in the inoculum and pathogenic for mice, they could have often been demonstrated in the peritoneal fluid in 5 to 10 days. Demonstration of *Toxoplasma* was the proof of infection. On the other hand, if an inoculated animal survived a month, a blood sample was taken and examined in the SFT; if the finding was positive, the animal was sacrificed and its brain searched for cystic forms of *T. gondii*, which also confirmed the diagnosis. If cysts were not seen, intraperitoneal subinoculation of a suspension of liver, spleen and brain into fresh mice was performed.

Finally, in the case of stillbirth, the autopsy finding enabled us to establish the diagnosis. The karyotype determination was carried out, wherever possible.

RESULTS

This study involved 21 pairs of twins; the diagnosis of congenital toxoplasmosis was established in 38 infants on the basis of immunobiological findings. As to the remaining four children, three were considered uninfected as *Toxoplasma* antibodies disappeared from their sera in several months, while one was macerated (this material was unfortunately not inoculated into mice).

The gestational age of all infants in our series was from 25 to 34 weeks and the birth weight of all was below 2 500 g. Both infants were of the same sex in 13 pairs (12 neonates were male and 14 female), of different sex in 7 pairs, and finally in one case one infant was male while his cotwin was macerated.

Out of the 21 pairs of twins, the karyotype was determined in 14 pairs (6 of them were homozygous and 8 heterozygous), and 7 pairs were not karyotyped.

Classical congenital toxoplasmosis in both twins was present in 5 pairs (cases 1, 4, 6, 10 and 11). The subclinical form of toxoplasmosis was present at birth in both twins of 3 pairs (cases 5, 7 and 8). The pattern of disease was the same in 6 homozygous pairs of twins (cases 1, 4, 5, 6, 7 and 8), but it was different in 8 heterozygous pairs (cases 2, 13, 14, 15, 16, 17, 18 and 20), as well as in cases 9, 19 and 21, who, although unkaryotyped, can be considered heterozygous as the children of a pair were of different sex. In two pairs one cotwin was macerated and stillborn, respectively (cases 3 and 12); in the cotwins born alive the diagnosis of toxoplasmosis was established by immunobiological methods. As mentioned previously, in 3 pairs of twins (cases 13, 14 and 17) clinical manifestations were present in one infant of a pair and one of these infected babies died (case 17), while the *Toxoplasma* antibodies disappeared from his cotwin's serum. In two pairs (cases 20 and 21), signs of disease which were not present at birth became manifest in one of the twins later on, while his cotwin appeared unaffected but still with a SFT strongly positive at the age of one (case 20) and four years (case 21).

Serological findings, type of karyogram and presence (or absence) of manifestations at birth are presented in chronological order in Figure 1. If toxoplasmosis was apparent at birth in at least one infant of a pair, both were followed serologically from the age of one month onwards.

Laboratory findings

Toxoplasma antibody levels detected by the SFT in neonates with clinical manifestations ranged from 1 : 100 to 1 : 8 000.

It was interesting that, among the three infants who were not infected, one had a positive SFT at the titre 1 : 1 000 in the neonatal period, and in two infants with no clinical manifestations the result of the SFT remained positive at high titres (1 : 1 000 and 1 : 4 000) at the age of one and four years, respectively.

We have also noticed that in most of the infants examined, the *Toxoplasma* IgG antibodies appeared to be at the lowest concentrations in the serum at the age of 3 to 5 months.

Antibody levels in the Remington test ranged from 1:2 to 1:100. The test was performed on 26 infants and it revealed IgM antibodies in 16 infants (61%). In 11 (42%) of them, the IgM antibodies were demonstrated already at birth, while in the other 5 (19%) the IgM antibodies became detectable in the first year of life. Among the 10 infants with negative Remington tests, 7 had signs of infection, the disease was latent in one child, and two were uninfected.

Out of the 23 attempts at the experimental isolation of the parasite, 4 were successful (cases 2, 3, 12 and 18).

Sixteen mothers of the observed infants were also examined serologically, their antibody levels ranging from 1:100 to 1:8000. Two of them had a history of moderate fever and malaise during pregnancy and one had enlarged lymph nodes.

DISCUSSION

It is well known that clinical features of congenital toxoplasmosis can vary very much (Couvreur and Desmonts 1962, Martinović et al. 1982). Among the children dealt with in this study, 20 neonates had no signs of disease at birth. Seven pairs of twins (cases 2, 9, 15, 16, 18, 19 and 21) had different clinical forms of the disease. In some cases, one infant of a pair died of congenital toxoplasmosis (cases 2, 15, 18 and 19), whereas his cotwin had a subclinical infection (isolation of the parasite was successful in one infant) and adequate therapy was applied in due time.

Both twins in all the homozygous pairs (cases 1, 4, 5, 6, 7 and 8) had the same pattern of disease: in three pairs, clinical manifestations were present already at birth, and in the other three pairs, the infection was subclinical. In all the heterozygous pairs (cases 2, 13, 14, 15, 16, 17, 18 and 20), the clinical pattern varied in each twin: one twin was uninfected in cases 13, 14 and 17, while in case 20, one infant appeared clinically unaffected despite positive results in the SFT. Among the 7 pairs whose karyotype was not determined, the infants in 3 pairs (cases 9, 19 and 21) differed in clinical manifestations. Thus, in case 9 signs of congenital toxoplasmosis were present at birth in one child and absent in his cotwin, in case 19 one infant succumbed to the disease while the other had a subclinical infection, and in case 21 one child had manifestations of the infection, while his cotwin had SFT results still positive at the age of four years, although he was clinically unaffected. In four pairs of unkaryotyped twins, all infants presented severe disease. Thus, in cases 3 and 12, one child was macerated or stillborn, while his cotwin died at the age of four and two months, respectively, and in cases 10 and 11, both infants of each pair had similar patterns.

One of our most interesting findings is that, although one infant in each of cases 13, 14 and 17 (heterozygous twins) was seriously infected (the infected infant in case 17 died), his cotwin was uninfected. This was proved by the disappearance of *Toxoplasma* antibodies by the time when two of them were 5 months and one one year old (this one was not controlled in the meantime).

Case 20 was followed until the age of one year, and case 21 until the age of four years. In both pairs, one infant had evident congenital toxoplasmosis, while his cotwin, despite strongly positive SFT results, had no clinical manifestations.

Our series included 6 homozygous twin pairs, 8 heterozygous pairs and 7 pairs of unkaryotyped twins. Both infants in all the homozygous pairs had similar pattern of disease, which is opposite to the finding in the heterozygous pairs. In the latter, the two infants of a pair had a very different degree of disorder: in three of our cases one child of a pair was not infected at all, while his cotwin was seriously infected. This finding is consistent with that by Couvreur et al. (1976) and others (Couvreur 1978, Murphy and Flannery 1952, Granstrom and Magnusson 1950), who concluded

that both infants in a homozygous pair are usually affected to the same degree: the type and localization of lesions are most frequently the same in both of them. On the contrary, in heterozygous twins with congenital toxoplasmosis, the disease pattern varies to such an extent that one infant of a twin pair may be uninfected, while his cotwin may have a serious disorder.

Since heterozygous twins are generally much more frequent than homozygous (Popović 1979) — 7:3, the chances are greater for both infants in a pair not to be equally infected. According to the findings of Couvreur et al. (1976) and to the ours, one infant in one third of heterozygous twins may escape infection. A possible explanation of this phenomenon is only one placenta. Nevertheless, we can conclude that in children born as twins, if the diagnosis of congenital toxoplasmosis has been established in one child, the other should be subjected to thorough clinical and serological examinations even if it appears unaffected, and, if necessary, adequate therapy should be applied as soon as possible.

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ВРОЖДЕННЫЙ ТОКСОПЛАЗМОЗ У НЕДОНОШЕННЫХ БЛИЗНЕЦОВ
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Резюме. При изучении проблемы „Токсоплазмоз и недоношенность“ исследовали 330 образцов крови близнецов. В работе приводятся результаты исследований 21 недоношенных близнецов (материнские сыворотки были также исследованы). Антитела против *Toxoplasma* были обнаружены при помощи реакции Сэбина—Фельдмана и специфические IgM антитела при помощи реакции Ремингтона. Классическая форма врожденного токсоплазмоза наблюдалась у пяти пар близнецов, тогда как у трех пар после рождения наблюдали субклиническую форму токсоплазмоза. У семи пар близнецов образ болезни был различным. У одного близнеца из двух пар встречались признаки болезни, тогда как другой близнец той же пары казался незараженным, но результаты реакции Сэбина—Фельдмана были сильно положительны. Однако самым интересным является факт, что из трех пар один близнец был явно заражен врожденным токсоплазмозом, тогда как другой близнец остался незараженным, что подтверждилось исчезновением антител против *Toxoplasma*. Это несомненно показывает, что для переноса инфекции важно, повреждена ли плацента или нет.

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N. N. Kuznetsov, V. M. Petrov: Khishchnye kleshchi Pribaltiki. (Predatory mites of Baltic republics). (Parasitiformes: Phytoseiidae, Acariformes: Prostigmata).

Publ. House Zinatne, Riga 1984, 144 pp., 105 Figs. Price 1.00 R.

The group of predatory mites includes some possible candidates for biological control of agricultural pests. The publication contains the main data obtained during the investigations of these mites on the territory of Latvia, Lithuania and Estonia. A 100-page systematical part follows after a short introduction, methodical chapter and survey of 141 collection localities. The text presents determination keys and characteristics of individual taxa. With the species, description of its female,

distribution on the territory of the USSR and that of the three Baltic republics are given accompanied by its figure. In total, 94 mite species belonging to 36 genera and 12 families are reported; among them 2 new species are described. The book closes by references (147 citations) and index of Latin names. It is a good base for further more detailed investigations of predatory mites on the given territory.

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