Abstract  A long-term follow up was begun in 1982 on offspring of mothers who acquired toxoplasmosis during gestation. The 114 newborns were subdivided into 3 groups: (1) 26 born to mothers with certain infection; (2) 51 born to mothers with probable infection, and (3) 37 born to mothers with doubtful infection. There were five infections in the first group (19.2%), three in the second (5.8%) and none in the third. For purposes of data elaboration we considered only the 77 offspring of mothers with certain or probable infection. Of these, 2 infected cases out of 52 (3.8%) were born to mothers with infection in the first trimester of pregnancy, 4 out of 21 (19%) in the second trimester, and two out of four in the third. There were a total of 8 congenital infections (10.4%). Specific IgM antibodies were revealed in five out of eight infected children (62.5%). Infection was symptomatic in two children (2.6% of newborns at risk, 25% of infected cases), both born to mothers with infection in the second trimester. In the other six cases diagnosis was reached by evaluating trends in antibody levels: the percentage of infected newborns was higher in the group of maternal infections untreated (50%) or improperly treated (15.4%), compared to those receiving adequate treatment (6.9%). We suggest considering as infected children presenting specific IgM antibodies and/or antibody titres which do not become negative, even when symptoms are absent. Therapy with spiramycin should be started in all newborns at risk, while the use of sulphamides and pyrimethamine is justified only after the presence of infection is confirmed.

Conclusion  Identification of susceptible women before or early in pregnancy would permit adoption of preventive measures aimed at reducing the frequency of congenital infection which is still high in our case series.

Key words  Congenital toxoplasmosis · Newborn · Pregnancy · Diagnosis · Therapy

Introduction  
Toxoplasmosis is a widespread infection which is benign in adults but may damage the fetus if acquired by the mother during pregnancy. In Italy, seropositivity for toxoplasmosis among women of childbearing age is 50%, with an annual seroconversion rate of 8.5%; thus approximately 6 out of 1000 women acquire the primary infection during pregnancy [1]. In the city of Turin, seropositivity in the general population was 87% in 1978 [9], 75.7% in 1981, and 45.5% of pregnant women in 1985 [12]. Using the Frenkel formula (1974), the annual risk of congenital
infection in Turin is estimated at 1.5% of live births, taking into consideration the annual seroconversion rate (0.5% yearly between 16 and 45 years), duration of pregnancy (0.75 = 75% of a year) and risk of infection for the offspring (0.4 = 40%) [8].

It is difficult to determine the moment when infection is acquired during pregnancy, since the immune situation of the mother before conception is rarely known, and since laboratory results often cannot be compared (having been performed in different laboratories, or not being expressed in international units). Furthermore, specific IgM antibodies are no longer considered a sure marker of recent or active infection.

Infection in the newborn is ascertainable only through laboratory tests, as clinical signs may be varied and non-specific or more frequently absent. It is important to identify infected newborns in order to begin therapy and thus prevent later manifestation of illness.

Based on these premises we evaluated: (1) whether in the last 10 years there has been any change in the medical approach to pregnant women and newborns: (2) what criteria should be used to identify newborns at risk based on the serological situation of the mother; (3) which newborns should be considered as infected and subjected to therapy; (4) how frequently infection is transmitted to the newborn, correlating time of infection and therapy administered to the mother.

### Materials and methods

We monitored newborns of mothers who acquired toxoplasmosis during pregnancy, identifying 114 newborns at risk of congenital infection over the course of 10 years, from 1 January 1982 until 31 December 1991.

Based on the results of maternal serological screening, children were divided into three groups:

1. Children born to mothers with seroconversion (certain infection).
2. Children born to mothers presenting antibody increase with high titres (ELISA > 300 IU, DA > 1/512, IFA > 300 IU), in the presence of specific IgM antibodies (probable infection).
3. Children born to mothers presenting stable medium-to-high titres (ELISA > 150 and < 300 IU, DA > 1/64 and < 1/512, IFA > 100 and < 300 IU), in the presence of specific IgM antibodies (doubtful infection).

Relative to the time of appearance of infection during pregnancy, another three groups of children were studied, born respectively to mothers with infection in the first, second and third trimester.

The monitoring scheme for newborns at risk provided for an initial serological check in the 1st week of life, allowing certain diagnosis in the presence of specific IgM antibodies. Therapy was started immediately in these cases even if they were asymptomatic. In cases where IgM antibodies were absent and infection could not be excluded, subjects were checked monthly in the first 3 months of life and then every 3 months until antibodies disappeared. Children who did not show a decrease in antibodies and did not become negative were considered infected, and underwent further serological and clinical tests once or twice yearly, depending on antibody response and appearance of symptoms. Newborns at risk also underwent a neurological examination, cerebral ultrasonography, examination of the fundus oculi, and a paediatric examination at the time of blood tests. Subjects suspected of infection underwent, at 6 months of age and thereafter every 6–12 months, a neurological examination and examination of the fundus oculi.

Blood samples were analysed using the following tests: IgG ELISA (ETI-TOXO K-G Sorin Biomedica standardized on International Standard Serum with results expressed as IU) and IgM ELISA (ETI TOXO K-M Sorin Biomedica which detects IgM antibodies anti-Toxoplasma with double-sandwich method until 1989 and since 1990 ETI TOXO K-M-Reverse which detects these antibodies with capture method); a direct agglutination test before and after treatment with 2-mercaptoethanol (TOXO AD COLOR-Bouty Diagnostici); indirect fluorescent antibody test (IFA Bio Mérieux reactives); IgM ISAGA (TOXO-IgM-ISAGA Bouty Diagnostici).

Before confirmation of infection, newborns were given 50–100 mg/kg per day of spiramycin, in two doses, repeated in 20-day cycles. In cases of highly probable or certain infection, specific therapy was administered using a commercial preparation of pyrimethamine-sulphadiazine at doses of one tablet (25 mg pyrimethamine + 500 mg sulphadiazine) per 20 kg every 10 days in asymptomatic subjects, one tablet per 10 kg every 7 days in symptomatic subjects, according to protocol suggested by Garin et al. [4]; the Italian commercial preparation contains sulphamethopyrazine instead of sulphadoxin: both are similar long-acting sulphamides. The classical treatment with pyrimethamine and sulphadiazine [2, 5] was not used because these drugs are not available in Italy. At the same time 4 mg/day of folic acid was given. Treatment was interrupted if blood counts showed platelets < 90.000/mmc, leucocyte levels < 4.000/mmc and Hb < 9g/dl. The treatment with sulphamides and pyrimethamine is continued for 12–18 months and interrupted usually when antibodies decrease. The appearance of symptoms or an increase in antibody rates of at least three dilutions indicates resumption of therapy for 4 months, as also recommended by the French authors [2, 4].

### Results

Of the 114 children examined, 26 were born to mothers with certain infection, 51 to mothers with probable infection and 37 to mothers with doubtful infection. Among these children the rate of infection was, respectively 19.2% (5/26), 5.8% (3/51), and none. For the purposes of statistical analysis we considered only the 77 children of mothers with certain or probable infection.

Serological analysis performed in the mothers showed the following results:

1. Infection occurred in the first trimester of pregnancy in 52 cases, in the second trimester in 21 cases, and in the third trimester in 4 cases; (2) in the first 5-year period of the study (1982–1986), seroconversion was found in only 7 out of 35 pregnancies (20%), while in the second 5 years (1987–1991) the number of seroconversions rose to 19 out of 42 pregnancies (45.2%); this difference is statistically significant ($P < 0.005$). Results were confirmed by correlating probable and certain infections with trimester of pregnancy and subdividing them by 5-year periods (Fig. 1); (3) the number of probable infections during the first trimester was high in both 5-year periods, whereas seroconversion was found in most cases in the second and third trimesters of pregnancy (Fig. 1).