

***Toxoplasma gondii* infection during pregnancy: a ten-year observation in the province of Trento, Italy**

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SUMMARY

The study analysed the trend of toxoplasmosis infection in pregnancy by using antenatal serological screening and the incidence of the congenital condition in newborns in the province of Trento, Italy. Data from pregnant women who gave birth at local maternity units between 2009 and 2018 were obtained. The serological test results were collected from birth attendance certificates (BACs), the main - and mandatory - source of information used to monitor pregnancies, births and neonatal health in Italy. The BAC used in the province of Trento acquires the results of serological tests for a vast range of infections in pregnancy. The data collected from the BACs were integrated with those provided by the Hospital Information System (HIS), which was also used to collect data on the trimester in which the seroconversion occurred. A total of 45,492 pregnant women were analysed, of whom 24% were foreigners. The average coverage of serological screening in pregnancy was 99.7%. Mean overall prevalence of *Toxoplasma gondii* infection was 21.7% (95% CI: 21.3-22.1): in Italians the prevalence was 17.9% (95% CI: 17.5-18.30) and in foreign nationals 32.7% (95% CI: 32.26-33.13). The mean annual seroconversion rate was 3.5‰ (95% CI: 3.0-4.2) of susceptible women and 2.7‰ (95% CI: 2.2-3.4) of all pregnant women who were screened. The seroconversion rate was higher amongst foreign

women (3.2‰, 95% CI: 3.0-3.6) than Italian women (2.4‰, 95% CI: 2.1-2.8). In all, 91.0% of seroconverted women were treated during pregnancy in accordance with the anti-toxoplasma protocol. Five cases of congenital infection were identified (2 amongst Italians and 3 amongst foreign women), amounting to an overall transmission rate of 4.0% (2.3% in Italians and 8.8% in foreigners). Transmission risk ranged from 0.0% in the first trimester to 19% in the third. The incidence of congenital toxoplasmosis, over the entire study period, was 0.12‰ live births (0.11‰ in Italians and 0.16‰ in foreigners). Data collection on infections in pregnancy through BAC allows area-based assessment. Although the quality of the data recorded in the BAC can be considered satisfactory, it was also necessary to access other information sources. The screening coverage was very high. The prevalence of toxoplasmosis infection was found to be higher in foreign mothers than in Italians, as well as seroconversion. The extent of serological screening and the high treatment rate helped to keep the risk of infection transmission to the foetus low and to achieve a very low rate of congenital infection.

Keywords: Pregnancy, birth attendance certificate, serological screening, toxoplasma infection, congenital toxoplasmosis.

■ INTRODUCTION

Toxoplasmosis is a very common infectious disease caused by *Toxoplasma gondii*, an obligate intracellular parasite that is extensively present in nature where cats, especially in western countries, represent the definitive host [1]. The

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disease is most frequently transmitted to humans by the consumption of raw or undercooked meat, or water, fruit and vegetables contaminated by the parasite's oocysts eliminated by the felines [2, 3]. Acquired infection in immunocompetent subjects usually has an asymptomatic course and resolves spontaneously. The infection contracted in pregnancy can result in congenital toxoplasmosis in the neonate, due to vertical maternal-foetal transmission. The likelihood of transmitting the infection to the foetus increases with gestational age, with a risk of around 2% in the case of infections contracted during the first trimester through to a risk of over 60-80% in those acquired during the third trimester [4, 5]. The diagnosis of acute infection in the mother should be made as swiftly as possible in order to promptly initiate therapy to reduce the risk of maternal-foetal transmission and the severity of congenital toxoplasmosis. A systematic serological screening in pregnancy can therefore be useful, although internationally there is disagreement on the appropriateness of such screening [6-10]. In Italy, the National Guidelines for the Management of Physiological Pregnancy issued in 2011 recommends tests for toxoplasma antibodies (IgG, IgM) for all pregnant women, to be done by the 13th week of gestation, followed by repeated tests every 30-40 days until delivery if the woman is not immune [11]. This study analyzed the trend of toxoplasmosis infection in pregnancy as identified by the antenatal serological screening amongst the pregnant women who gave birth in the maternity units of the province of Trento, Italy from 2009 to 2018 and the incidence of the congenital condition in neonates.

■ MATERIALS AND METHODS

Serological screening for *Toxoplasma gondii* infection was carried out in all maternity units of the province of Trento, according to the protocol of the national multidisciplinary technical group [12]. The screening kit used was Abbot's Alinity reagent that is based on a chemiluminescent microparticle immunoassay. The cut-off value for IgG positivity is ≥ 3.0 IU/mL, and the cut-off value for IgM positivity is ≥ 0.60 Index. In the case of seroconversion during pregnancy and/or in the presence of IgM positivity at the first test, an avidity test was administered to confirm maternal infections with values $\leq 25\%$. An amniocentesis with PCR analysis of the presence of the parasite was offered to all pregnant mothers with confirmed positivity. The amniocentesis was performed no earlier than the 18th week of pregnancy, but at least 4-6 weeks after maternal infection. If the infection was contracted before the 20th week of pregnancy, the woman was treated with spiramycin 3 g/day for her entire pregnancy; if the infection was contracted after the 20th week of pregnancy, a combination of pyrimethamine 25-50 mg/day, sulfadiazine 3 g/day and folic acid 25-50 mg twice a week was prescribed [11, 12]. All neonates born to mothers who seroconverted during pregnancy were undergone serological monitoring with specific IgG, IgM and IgA tests. A Western Blot test was also performed on a sample from the mother and child for the typing of the IgG and IgM. A diagnosis of congenital toxoplasmosis in absence of objective clinical signs and symptoms was usually formulated when the result of the specific IgG test at birth was >250 IU. Newborns were followed up with the following protocol: clinical evaluation at birth and at 1, 2, 3, 6, 12 and 24 months of age, with periodic instrumental examinations such as cerebral ultrasound, vision and hearing examination. The results of the mother's serological tests were entered in the personal antenatal guide of each pregnant woman and recorded by the midwife present at birth when the Birth Attendance Certificate (BAC) was drawn up and electronically filed. The BAC is the main source of information used to monitor pregnancies, births and neonatal health across Italy [13]. The BAC used in the province of Trento has recorded since the early 2000s a greater number of variables than are required by the ministerial template. More specifically, the variables acquired include the results of the serological tests for a vast range of infections affecting pregnancy. Serological tests related to toxoplasma infection are recorded in the BAC according to the following criteria:

a) test not performed,
 b) non-immune,
 c) immune,
 d) test in progress,
 e) seroconversion in pregnancy.

We analyzed the data from pregnant women who gave birth at local maternity units between 2009 and 2018. For cases recorded as "test in progress" we retrospectively retrieved the data by accessing

the Hospital Information System (HIS), a repository that contains the results of all examinations, hospitalisations and diagnostic tests for users of healthcare services in the province of Trento, Italy. The HIS was also used to get the data for seroconverted women in order to estimate the trimester of seroconversion. We calculated the coverage of serological screening for each year, and in relation to the mother's nationality, age group and academic qualification; the infection prevalence for all pregnant women, and in relation to the mother's age group, nationality and academic qualification; the seroconversion rate for the entire caseload, and in relation to the mother's nationality, age group and academic qualification. Time trend significance was analyzed using the Cochran-Armitage criterion, and the significance of the differences between the compared categories were analyzed using the Chi-squared test and Fisher's exact test. The appropriate 95% confidence intervals (95% CI) were calculated for each prevalence value. The health of neonates born to seroconverted mothers was analyzed through these information sources: the neonatal data recorded in the BAC, hospital discharge records and the serological profile (toxotest) up to 12th months of age.

RESULTS

A total of 45,492 pregnant women were analyzed, of whom 95% were resident in the province of Trento, Italy. 75.5% were Italians (average age 33.0 years) and 24.5% were foreign (average age 28.7 years). The proportion of foreign pregnant wom-

en rose from 23.3% in 2009 to 26.3% in 2018. The corresponding number of live births for the same period was overall 48,059. The average coverage of serological screening in pregnancy was 99.7% and was consistently higher than 99% throughout the study period (Figure 1). No statistically significant differences in coverage were observed in relation to nationality, age and education level. Mean overall infection prevalence in pregnant women was equal to 21.7% (95% CI: 21.37-22.13); amongst Italians it was 17.9% (95% CI: 17.5-18.30) and amongst foreigners it was 32.7% (95% CI: 32.26-33.13), with a statistically significant difference ($p < 0.001$). The time trend of the infection prevalence decreased (Figure 2) significantly amongst both Italian and foreign women ($p < 0.01$). Amongst foreigners seroprevalence was higher in mothers from Latin American countries and lower in Asian women, with a statistically significant difference ($p < 0.01$) compared to the mean values for foreign women (Figure 3). No differences in seroprevalence were observed in relation to age and education level. The total number of cases of seroconversion, confirmed by the HIS, was 123 (34 of which in foreign mothers), equal to 12.3/year. The data acquired from the HIS confirmed approximately 95% of the original data recorded in the BAC. The mean annual seroconversion rate was 3.5‰ (95% CI: 3.0-4.2) of susceptible women and 2.7‰ (95% CI: 2.2-3.4) of all pregnant women who were screened (Figure 4). Considering the total number of pregnant women tested, the seroconversion rate was higher in foreign women (3.2‰, 95% CI: 3.0-3.6) than amongst Italian

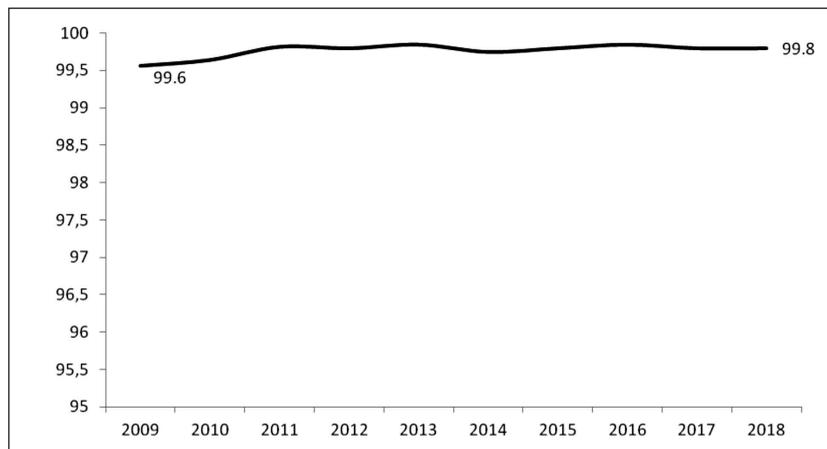


Figure 1 - Province of Trento. Serological screening coverage for Toxoplasmosis Infection in pregnancy. Trend 2009-2018.

Figure 2 - Province of Trento. Seroprevalence of *Toxoplasma gondii* infection in pregnancy. Overall and by citizenship. Trend 2009-2018.

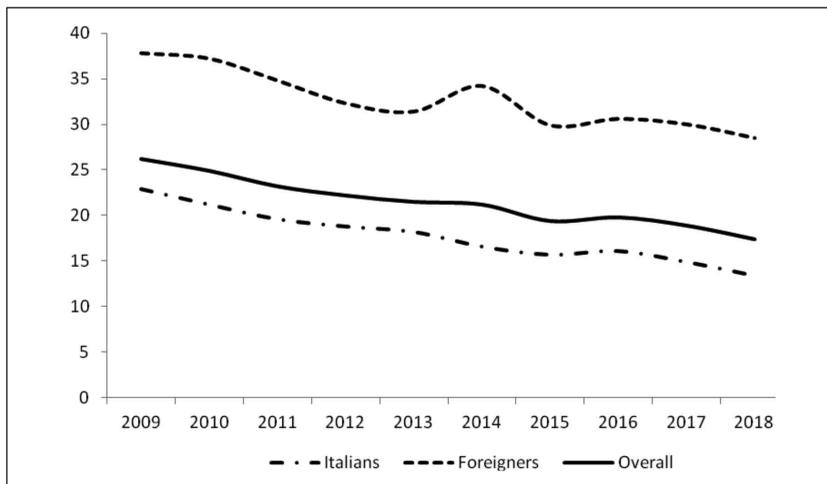


Figure 3 - Province of Trento. *Toxoplasma* infection in Pregnancy - Seroprevalence/1000. By geographical area of origin of the pregnant women. Period 2009-2018.

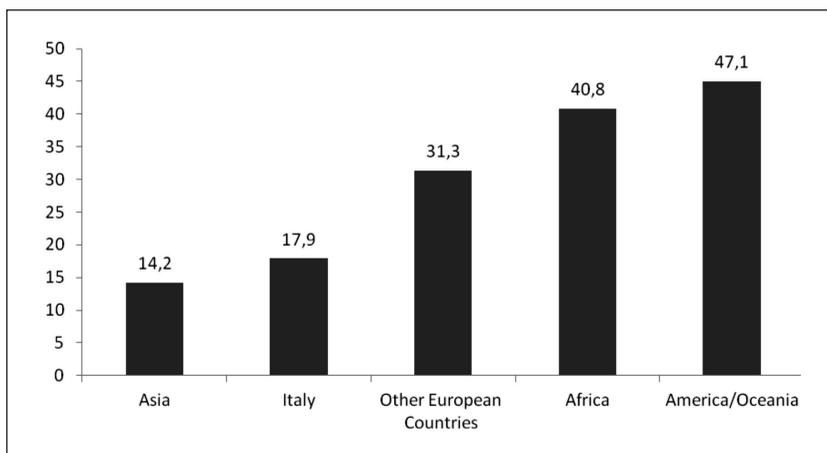
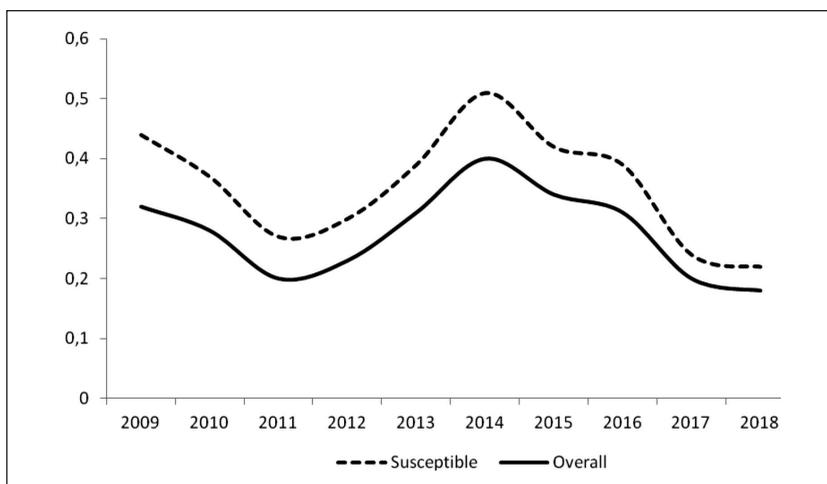


Figure 4 - Province of Trento. Seroconversion rate of *Toxoplasmosis* infection (per 1000) in susceptible pregnant women and in overall tested pregnant women. Period 2009-2018



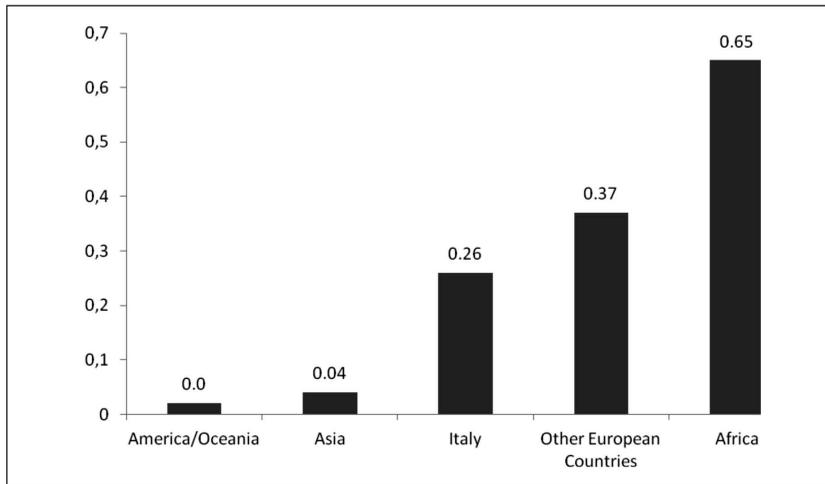


Figure 5 - Province of Trento. Seroconversion rate for Toxoplasma infection in pregnancy (per 1000). By geographical origin of pregnant women. Period 2009-2018.

women (2.4%, 95% CI: 2.1-2.8) with a statistically significant difference ($p < 0.05$). Amongst foreign women, the seroconversion rate was higher in women from African countries, with a statistically significant difference ($p < 0.01$), than the average value for foreign women (Figure 5). No statistically significant differences in seroconversion were observed from one year to another or in relation to age group or academic qualification. The consultation of the HIS made it possible to identify the period in which seroconversion occurred in 121 of the 123 pregnant women (98.4%). In evaluable women, it was observed that, 55.3% experienced seroconversion in the first trimester, 27.3% in the second trimester and 17.3% in the third trimester. The foreign women seroconverted more frequently in the first trimester than the Italian women (57.3% vs 50.5%), although this difference is not statistically significant. 56% of seroconverted women had an amniocentesis, with a higher rate in Italian women (58%) than in foreign women (42.3%), although, once again, this difference was not statistically significant. The amniocentesis was positive in just 2 cases. 91.0% of seroconverted women were treated during pregnancy in accordance with the protocol, without any difference between the foreign and the Italian women. All neonates born to seroconverted mothers (123) were live births and we were able to retrieve the data for the serological monitoring performed in the first year for 120 cases out of 123 babies (97.5%). An average of approximately 4 toxo tests was performed during the first year of life; 75 ne-

onates presented IgG positivity at birth and 3 IgM positivity at birth (in addition to IgG positivity). Using lab test results and the data from other diagnostic procedures, 5 cases of congenital infection were identified, 2 amongst Italian women and 3 amongst foreign women, for an overall transmission rate of 4.0%; respectively 2.3% amongst Italian women and 8.8% amongst foreign nationals. The risk of transmission was seen to be null in the case of infection in the first trimester, 6% for infection in the second trimester and 19% for infection in the third trimester. The incidence of congenital toxoplasmosis, over the entire study period, was 0.12‰ live births: 0.11‰ amongst Italian women and 0.16‰ amongst foreign nationals.

■ DISCUSSION

The availability of monitoring data regarding Toxoplasma infection in pregnancy, using a current data flow such as that of the BAC database, makes possible to perform assessments that are not so much "centre-based" as primarily "area-based". Indeed, this study, probably one of the largest of its kind to have ever been conducted in Italy, considered practically all the pregnancies followed at the maternity units of the province of Trento, Italy. This opportunity is guaranteed by the implementation of a BAC template that involves the acquisition of a far greater number of variables than that present in the "ministerial" template and the transmission of the annual computerized database to the

Clinical and Evaluation Epidemiology Service of the Provincial Health Authority of Trento. The quality of the data recorded in the BAC can be considered on the whole to be satisfactory, although it was also necessary to access other information sources in order to retrieve the results of the tests in progress, performed close to the birth, for which the results were not available for recording before the BAC was signed off. In this study, the screening for *Toxoplasma* infection in pregnancy involved almost all the pregnant women who gave birth in maternity units of the province of Trento, without differences in terms of age group, academic qualifications and nationality. The Italian regulatory system, which provides free serological screening, has undoubtedly helped to guarantee widespread and equal access to prenatal screening [11,12]. The coverage of screening in our province would appear to be higher than that reported by previous Italian studies [14-18]. The prevalence of Toxoplasmosis infection in pregnancy varies greatly in the various worldwide populations [19, 20] and our findings would appear to be in line with previous Italian studies [14-18, 21]. Seroprevalence was consistently higher amongst foreign women than amongst Italian women and decreased over time in both groups. This decrease, which was also described previously, is thought to be the result of changes in eating habits and improved hygiene practices during pregnancy [6, 22]. Foreign women originating from Central and South American countries present greater seroprevalence, in accordance with the findings reported in literature [19, 20]. It is likely that the different seroprevalence values of infection reported in specific geographical areas of Italy are influenced by the rate of external migration and by the different nationalities of origin [19]. Seroconversion during pregnancy is a rare event and regards on average 3.5 cases per 1000 susceptible pregnant women and 2.7 cases per 1000 pregnant women undergoing screening, values that would appear to be slightly higher than those reported in studies conducted in northern European countries, but largely consistent with the findings of previous Italian studies [15-18, 23-26]. Seroconversion did not present any statistically significant variations from one year to another, despite the presence of a slight downward trend. The seroconversion rate was higher amongst foreign than

among Italian women and foreign women from African countries presented a higher seroconversion rate. This should stimulate an in-depth study of living conditions in populations at greatest risk and the implementation of actions promoting the adoption of healthier lifestyles and eating practices during pregnancy [15, 17, 25]. The overall incidence of congenital toxoplasmosis, over the entire study period, was 0.12‰ live births: 0.11‰ amongst Italian women and 0.16‰ amongst foreign nationals, respectively. On the whole, the incidence appears to be lower than reported in the available Italian studies and in reviews of international studies [16-18, 20, 25, 27]. The efficacy of systematic screening for *Toxoplasma gondii* infection during pregnancy is still a topic for debate, considering that there is no general agreement on the efficacy of antitoxoplasma medical therapy on vertical transmission to the fetus [7, 22, 28]. The implementation of primary prevention actions, through hygiene and dietary practices that are potentially efficacious for preventing infection in the mother, could be considered a sufficient measure [22, 29]. These measures alone may not be sufficient in women at greater risk such as foreign women. On the other hand the WHO nevertheless recommends thorough epidemiological data acquisition regarding toxoplasmosis infection in pregnancy [30]. The purpose of serological screening is to identify early and treat pregnant women who contract the infection in pregnancy [12]. A timely identification of maternal infection makes possible to plan ultrasound follow-up, perform an amniocentesis to investigate the possible mother-to-child transmission of the infection and consider the potential treatment options (treatment of the infection *in utero*, medically-indicated termination). With the current treatment options, at least 90% of babies with congenital toxoplasmosis are born without clinically-overt symptoms and are found to be negative during routine paediatric check-ups. In our experience, antenatal screening made possible to identify infection in a timely manner and initiate prophylactic treatment in order to prevent vertical maternal - foetal transmission that, as was confirmed at birth, only involved 5% of cases. More specifically, treatment was seen to be efficacious in preventing the risk of transmission in those cases in which infection took place during the third trimester of pregnan-

cy, which could justify universal screening. In any case, only the long-term monitoring of the neonates will make possible to confirm its efficacy regarding the risk of developing ocular and neurodevelopmental sequelae.

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Conflicts of interest

None

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None

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