SHORT REPORT
A 4-year evaluation of toxoplasmosis seroprevalence in the general population and in women of reproductive age in central Italy

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SUMMARY
This study aimed to estimate possible changes in seroprevalence of anti-Toxoplasma gondii IgG and IgM antibodies in people living in the area of Massa and Carrara (central Italy), in recent years. Serum samples from over 13 000 individuals were tested for both IgG and IgM anti-Toxoplasma antibodies using an immunoenzymatic method (Access® Toxo IgG, and Access® Toxo IgM II, Beckman Coulter Inc., USA). Our survey showed a decreasing trend of overall seroprevalence of 24.4% [95% confidence interval (CI) 22.62–25.71] in 2010 compared to 31.0% (95% CI 29.29–32.72) in 2007. A positive trend according to age was found, with low positivity observed in younger age groups. For women of reproductive age the prevalence of IgG antibodies was 30.2% (95% CI 28.44–31.96) in 2007 and 23.6% (95% CI 22.05–25.20) in 2010. IgM seroprevalence in women of this age group also progressively decreased from 1.6% to 0.97% during the study period. Our study confirms a decline of toxoplasmosis in Western countries.

Key words: Seroprevalence, specific IgG and IgM, Toxoplasma gondii.

INTRODUCTION
Toxoplasmosis is one of the most frequent parasitic infections in humans, caused by the coccidian parasite Toxoplasma gondii [1]. This protozoon is able to infect various warm-blooded animals, including humans, and birds [1]. Human toxoplasmosis is usually subclinical or presents with non-specific symptoms, e.g. fever, asthenia. In immunocompetent individuals typical manifestations include lymphadenopathy and chorioretinitis, and when infection is acquired during pregnancy the foetus may be severely affected. Poor outcome characterizes infection acquired by immunocompromised individuals [1].

Some studies have shown a progressive decline in seroprevalence in the last two decades in developed countries both in USA [2] and the European Union [3–5].

In Italy, due to the lack of a nationwide epidemiological surveillance system [6, 7], there is no epidemiological data about the seroprevalence of T. gondii in the general population and only sparse data exist about seroprevalence of T. gondii in pregnant women [8, 9]. The trend in pregnant women in

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Italy is in agreement with worldwide epidemiology; however, it should be noted that although the prevalence of toxoplasmosis is declining in many countries, the incidence of infection remains high [8].

Our study aimed to estimate the trend in seroprevalence of *T. gondii* infection in individuals living in central Italy, over a 4-year period, in order to evaluate the trend of exposure to the parasite. In addition, the study focuses on the actual prevalence of anti-*T. gondii* IgG in women of reproductive age in order to activate preventive measures, and in young people to understand future epidemiological trends.

Serum samples from 13,177 subjects (11,711 females, 1,466 males) aged from 12 months to 89 years (mean age ± s.e.m. 36.11 ± 0.19 years), living in the Massa Carrara area, North West Tuscany (central Italy), and presenting for serological tests at sample collection points distributed in different districts of the Azienda Sanitaria Locale 1 (ASL 1) (10,733 outpatients), and inpatients hospitalized in Massa, Carrara and Fivizzano, and Massa-Carrara (2,444 inpatients) hospitals, mainly in the departments of Obstetrics & Gynaecology, Infectious Diseases, Paediatrics, Ophthalmology, and Neurology. Samples were analysed for both IgG and IgM anti-Toxoplasma antibodies. For this study, we used data that had been collected in the period January 2007 to December 2010.

IgG and IgM anti-Toxoplasma antibodies were detected by immunoenzymatic methods (Access® Toxo IgG, and Access® Toxo IgM II; Beckman Coulter Inc., USA). Samples were considered positive for IgG concentrations ≥ 10.5 IU/ml, undetermined for IgG concentrations between 10.5 and 7.5 IU/ml, and positive for IgM concentrations ≥ 1 IU/ml; those with concentrations between 1 and 0.8 IU/ml were classified as undetermined. The IgM-positive samples were subsequently tested by means of an enzyme-linked fluorescent assay (ELFA; Vidas Toxo IgM, bioMérieux, France) for confirmation, and were considered positive when the index was ≥ 0.65. The specificity and sensitivity of the Vidas ELFA were 99.25% and 96.0%, respectively.

Prevalence data (percentage of subjects with a positive test) are given with 95% confidence intervals (CIs). Data from multiple tests were deleted from statistical analysis. SAS v. 8.2 software (SAS Institute Inc., USA) was used to perform statistical data analysis. The χ² test was used to evaluate the effects of sex and age on the immunological status of patients. The differences were considered statistically significant when *P* < 0.05.

Assuming independence between number of occurrences and time intervals, a Poisson regression was applied for modelling the logarithm of count of IgG + or IgM + as a linear function of observed covariates (sex and year). Each covariate was considered as a category variable; male gender and the year 2007 were considered as reference levels.

In total, serum samples from 13,177 subjects (11,711 females, 1,466 males) were tested for both IgG and IgM antibodies against *T. gondii* (Table 1). The number of individuals was homogeneous for each year, 3,150 in 2007, 3,346 in 2008, 3,384 in 2009, and 3,297 in 2010. Table 1 shows the distribution of individuals according to year and gender.

Most (10,685/13,177, 81.1%) serum specimens were from individuals referred to external sample collection points for routine tests. Overall, a total of 3,626 subjects, corresponding to 27.5% of the sample, were found to be positive for IgG antibodies (95% CI 26.7–28.3). A significant (*P* < 0.0001) difference in IgG seroprevalence in men (35.3%, 95% CI 32.89–37.78) and women (26.5%, 95% CI 25.74–27.34) was found.

Our data (Fig. 1) show a significant (χ² = 32.408, *P* = 0.0001) decline in IgG prevalence in females in
2008 (25·5%, 95% CI 23·96–27·10) compared to the previous year (31·0%, 95% CI 29·29–32·72 in 2007) which remained low across the other years (25·7%, 95% CI 24·14–27·28 and 24·2%, 95% CI 22·62–25·71 in 2009 and 2010, respectively). Conversely, a positive trend of IgG seroprevalence was observed in men, with values ranging from 32·8% (95% CI 27·88–37·66) in 2007 to 37·4% (95% CI 32·24–42·65) in 2010; however, because of the small number of individuals the difference did not reach significance. IgM antibody was detected less frequently than IgG with a total of 217 subjects testing positive (1·65%, 95% CI 1·43–1·86).

Results using Poisson regression for modelling the positivity to IgG, estimated a significant 29% decrease from 2007 to 2008 (P < 0·025), that became 6% from 2008 to 2009 (not significant) and significant again (28%) from 2009 to 2010 (P < 0·03). Females showed a 21% higher positivity for IgG than males. No significant differences were found in IgM counts.

Female subjects were then stratified into three age groups (1–15, 16–45, >46 years) and IgG and IgM seroprevalences were evaluated in each age group, both for the whole period and in each of the four individual years. A significant positive correlation (P < 0·0001) for IgG seropositivity with age was observed, with values ranging from 3·98% (95% CI 2·01–5·95) in the younger age group to 68·0% (95% CI 63·39–72·74) in older women.

The overall prevalence for anti- *Toxoplasma* antibodies for women of reproductive age (16–45 years) was 25·9% (95% CI 25·05–26·69) for IgG and 1·31% (95% CI 1·09–1·52) for IgM, respectively. A significant (P < 0·0001) reduction over time of IgG seroprevalence in females of the 16–45 years age group was also observed. In particular, IgG seroprevalence decreased from 30·2% (95% CI 28·44–31·96) in 2007 to 24·9% (95% CI 23·32–26·54) in 2008 and 23·6% (95% CI 22·05–25·20) in 2010. A decreasing trend was also observed for IgM from 1·6% in 2007 to 0·98% in 2010 (P = 0·036).

By analysing the distribution of inpatients, according to hospital departments, it is clear that most requests for a test came from the departments of Paediatrics (14·9% of inpatients), Infectious Diseases (14·0%), and Obstetrics & Gynaecology (12·1%). Only a small percentage came from the departments of Ophthalmology and Neurology (1·6% and 0·8%, respectively). No significant difference of this distribution was observed during the 4-year period (data not shown).

As already stated, in Italy a nationwide epidemiological surveillance system for toxoplasmosis (either congenital or not) is lacking. Although surveillance (on living new babies and pregnant women) is available, but only on a regional basis (e.g. Campania Region) [6]. As a consequence, it is difficult to retrieve up-to-date epidemiological data on the prevalence of *Toxoplasma* infection in the general population and on the incidence of congenital toxoplasmosis.

A declining trend in seropositivity both in women of reproductive age and during pregnancy, has become evident over the two decades in several EU countries [3, 5, 10, 11] as well as in the USA [2]. In Italy, a decreasing trend has been observed since 2001 in women of reproductive age [8], but data on toxoplasmosis seroprevalence in pregnant women are limited and there are few studies on seroprevalence of the infection in the general population [8, 9, 12, 13]. However, there was no decrease in the incidence of toxoplasmosis in pregnant women, which is still relatively high [3–5, 8–10].

Our main objectives were to evaluate the current status of *Toxoplasma* seroprevalence in inhabitants resident in central Italy, and to monitor the epidemiological situation in our area over the time. Our study showed a decreased rate of anti-*Toxoplasma* IgG seroprevalence from 31·0% to 24·4% over a 4-year period in individuals living in the urban area of Massa and Carrara, in agreement with previous results [14]. Such a decrease was also observed in women of reproductive age (15–45 years).

On the contrary, the trend in men of all ages appears different from one year to another. However, since data do not differ in a statistical way, evaluation of higher numbers of individuals is necessary to confirm the trend. The reduction of seropositivity for *T. gondii* is of great interest in women of reproductive age because of the risk of contracting primary infection during pregnancy.

When analysing data of the distribution of inpatients for which a test was requested, it is important to note that possible symptomatic cases did not follow the declining trend of seroprevalence observed during the 4-year period in the whole population examined. As expected, the departments from which requests were most frequently received were Infectious Diseases, Paediatrics and Obstetrics & Gynaecology.

The present study has some limitations because data were collected retrospectively and a bias in the selection of ‘patients’ is possible, since it included
mostly individuals referred to sample collection points distributed in different districts.

In conclusion, our work is the first survey performed in central Italy, during a 4-year period, and provides an opportunity to highlight the importance of national and regional surveillance systems in order to implement interventions aimed at the continued collection of data on toxoplasmosis seroprevalence over time, both in the general population and in particular, in women of reproductive age.

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DECLARATION OF INTEREST
None.

REFERENCES