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The rural–urban effect on spatial genetic structure of type II *Toxoplasma gondii* strains involved in human congenital toxoplasmosis, France, 2002–2009

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ABSTRACT

Congenital toxoplasmosis involves *Toxoplasma gondii* type II strains in 95% of cases in France. We used spatial principal component analysis (sPCA) and 15 microsatellite markers to investigate the spatial genetic structure of type II strains involved in 240 cases of congenital toxoplasmosis in France from 2002 through 2009. Mailing addresses of patients were geo-referenced a posteriori in decimal degrees and categorized into urban or rural areas of residence. No spatial genetic structure was found for type II strains that infected mothers who were living in urban areas, but a global spatial genetic structure was found for those that infected mothers who were living in a rural environment. Our results suggest that sources of infection by *T. gondii* are different in rural and urban areas in France, and advocate for targeted messages in the prevention of toxoplasmosis according to the type of residence of susceptible people.

1. Introduction

Toxoplasma gondii is considered to be one of the most successful parasites because this protozoan is virtually able to invade any nucleated cell of any warm-blooded animal at all latitudes. This parasite is efficiently transmitted by ingestion of oocysts shed by felids in the environment or cysts present in tissues of mammalians and birds. Toxoplasmosis is considered a benign disease except in fetuses infected during early pregnancy and in severely immunocompromised patients because they are at risk of severe brain and eye damage, and in tropical South America where the prevalence of ocular disease is high and where life-threatening cases of pulmonary toxoplasmosis may occur in otherwise healthy people (Carme et al., 2009; de-la-Torre et al., 2013). It is estimated that 25% of the human population is currently chronically infected by *T. gondii*. In fact, seroprevalence in the general population can tremendously vary with geography and also with time. Less than 10% of people born in the USA have antibodies against *T. gondii* whereas in certain tropical areas of South America and Africa, the prevalence is as high as 80% (Krueger et al., 2014). Big variations of prevalence can also be observed at a country scale. For example in France, the median prevalence among women of childbearing age was 43.8% in 2003 but the prevalence varied substantially by French region, being lowest in North-eastern France (29.5%) and highest in the greater Paris area (52.7%) (Berger et al., 2009). Seroprevalence varies also with time as shown in France with a decrease in prevalence from 83% in 1965 to 37% in 2010 (Nogareda et al., 2014). These data indicate that the epidemiology of

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human toxoplasmosis is complex and involves many different aspects including intensity of *T. gondii* circulation in the environment, geoclimatic variables such as temperatures, precipitations or altitude, and human factors such as personal hygiene, cultural and food habits, socio-economic status, level of education and residence in a rural or urban area.

The population structure of this cosmopolitan parasite is more complex than initially thought and has distinct geographic patterns. The hotspot of *T. gondii* genetic diversity seems to be tropical South America, especially in the Amazonian forest, where a combination of a large gene pool with frequent genetic exchanges have generated a wide variety of so-called atypical genotypes (Ajzenberg et al., 2004). Elsewhere, and especially in Europe, *T. gondii* displays a striking clonal population structure. Based on genotyping data obtained from domestic and wild animals in France, it is estimated that 99% of *T. gondii* strains that circulate in the French environment belong to type II (Aubert et al., 2010; Halos et al., 2010). Type III strains are much less frequent in animals in France than in animals from other areas such as North America or some regions of Africa (Shwab et al., 2014). To date, type I and atypical strains have never been isolated from any animal species in France. As a consequence, isolating a non-type II strain in a patient in France raises the possibility that this patient had been infected with an imported strain during a journey abroad or because he had eaten imported food, as already shown in some reports (Ajzenberg et al., 2009; Pomares et al., 2011).

The aim of this study was to better understand the epidemiology of human toxoplasmosis in rural versus urban areas by investigating the spatial genetic structure of *T. gondii* strains in both environments at a country scale in France. If people who live in rural areas are predominantly infected by local food produced in their region of residence, then a significant spatial genetic structure of *T. gondii* strains should be observed in rural areas. Conversely, if we consider that people living in urban areas are more likely to get *T. gondii* infection from food purchased from supermarkets, no spatial genetic structure should be observed in urban areas because goods distributed in supermarkets come from many different suppliers all over the French territory. To test this hypothesis, we used strains isolated in France from congenital cases because these strains are predominant in the collection of human strains at the French national reference center of toxoplasmosis and because women are infected during a short period of time during pregnancy, which minimizes the likelihood of *T. gondii* infection outside their residency area.

2. Material and methods

2.1. T. gondii strains

From 1992 to 2009, the French national reference center of toxoplasmosis genotyped 325 *T. gondii* strains responsible for congenital toxoplasmosis in 16 French teaching hospitals. These strains had been isolated in mice after inoculation of amniotic fluids, placenta, or blood cord samples for evidencing congenital toxoplasmosis in children or fetuses from mothers infected by *T. gondii* during pregnancy in this period. All these strains were genotyped with 15 microsatellite markers as previously described (Ajzenberg et al., 2010). Of these 325 strains, 305 (94%) were type II, 10 (3%) were type III, and 10 (3%) could not be classified into one of the three major clonal types and therefore considered as atypical.

For the analysis, we restricted the sample to the strains isolated during a shorter period of time, from 2002 through 2009, in order to minimize temporal effect on genetic variability. We also excluded the type III and atypical strains in order to minimize the genetic variability due to imported strains from non-European countries. In total, 240 type II strains isolated from 240 cases of congenital toxoplasmosis in France from 2002 through 2009 were included in the analysis (supplementary material).

2.2. Geo-referencing T. gondii strains in France

The 240 *T. gondii* strains were geographically associated to the place of residence of the corresponding mothers in whom these strains had been collected. The mailing addresses (number, street and commune names) of the 240 patients were geo-referenced *a posteriori* in decimal degrees using the web portal of the French National Institute of Geography (IGN, http://www.geoportail.fr/). The spatial distribution in France of the 240 strains is displayed in Fig. 1. Some gaps can be identified in this map. The big gap in the center of the country is mainly due to the fact that this area (called Massif Central) is the least densely populated area in France. Other gaps in certain densely populated areas are explained by the poor availability of strains from these areas at the French national reference center of toxoplasmosis.

2.3. Classification of T. gondii strains into rural and urban

In order to investigate the relative degree of spatial genetic structure of *T. gondii* strains isolated from women living in a rural environment versus those living in an urban environment, the geographic coordinates of the 240 strains were classified as rural or urban based on two different classifications.

2.3.1. TUU classification

A detailed classification of the 36,569 French communes within rural versus urban socio-economic environments was delivered in 1999 by the French National Institute for Statistics and Economic Studies (INSEE, available at http://www.insee.fr/en/default.asp) and updated in 2009. This classification is based on urban unit sizes ("Taille d'Unité Urbaine" or TUU in French) and includes several criteria such as population size, socio-economic activity within the commune, or frequency of travel between place of residence and work. Based on the TUU classification, the rural communes are therefore those that are not urban. The TUU classification allowed us to classify n = 85 *T. gondii* strains as rural and n = 155 *T. gondii* strains as urban (supplementary material).

2.3.2. GE classification

The TUU classification can be completed by another one that classifies urban communes as those that are not rural. We used the Google Earth (GE) application (http://earth.google.com/) to display a satellite windows (10 km × 10 km) centered on the mailing addresses of the 240 patients. When no commune of more than 5000 inhabitants was found at less than five kilometers of a given mailing address, the *T. gondii* strain isolated from this given patient was classified as rural. A strain was thus classified as urban when the previous condition was not met. Overall, it allowed us to classify n = 98 *T. gondii* strains as rural and n = 142 *T. gondii* strains as urban (Fig. 1 and supplementary material). Matching between the TUU and GE classifications was very good since 79 out of the 85 rural *T. gondii* strains that were defined with the TUU classification were also rural with the GE classification (observed Cohen's unweighted Kappa coefficient = 0.78).

2.4. Genetic and genotypic diversity within rural and urban populations

Genetic polymorphism was measured by allelic richness (A) per locus and sample and by Nei's unbiased genetic diversity within subsamples (Hs) (Nei and Chesser, 1983). Allelic richness was corrected for unequal sample size using the rarefaction method (El Mousadik and Petit, 1996). The A and Hs values were calculated with the FSTAT software version 2.9.4 (Goudet, 1995). Genotypic diversity was calculated from the number of multilocus genotypes on the total number of strains for both rural and urban populations. Linkage disequilibrium (LD) between pairs of loci was assessed with a randomization test performed in FSTAT. The statistics used was the log likelihood ratio G summed over all subpopulations. Because this procedure was repeated on all pairs of polymorphic loci, we applied the sequential Bonferroni