New description of Toxoplasma gondii genotypes from French Polynesia

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ABSTRACT

We report here the first isolation and genotyping of two human Toxoplasma gondii strains from French Polynesia. The parasites had new and atypical genotypes, and were responsible for asymptomatic congenital toxoplasmosis. Both genotypes were divergent from the common strains isolated in Europe, North America, South America, Africa and China.

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1. Introduction

In North America and Europe, Toxoplasma gondii displays a population structure consisting of few major clonal lineages. Type II strains are predominant in Europe, whereas in North America, type 12, II and III account for the majority of strains (Ajzenberg et al., 2002; Dubey et al., 2011; Elbez-Rubinstein et al., 2009). However, sampling from South America and sub-Saharan Africa has revealed that strains from these regions are highly divergent (Ajzenberg et al., 2009, 2004; Demar et al., 2007; Pena et al., 2008). The parasite genotype may play a role in the severity of the infection in immunocompetent patients, and in congenital infections (CT) (Delhaes et al., 2010; McLeod et al., 2012). Atypical strains other than type II strains have been reported with a higher virulence in CT. The clinical outcome of CT, is variable, ranging from subclinical (retinochoroiditis may occur at birth or later during childhood) to severe (fetal death, spontaneous abortion and severe neuro-ophthalmic involvement). The main factor determining the severity of CT is the gestational age at the time of fetal infection based on data collected in France. A fetal infection in early pregnancy has more severe consequences than an infection in late pregnancy (Dunn et al., 1999).

In this study, we report the isolation and genotyping of two T. gondii strains from French Polynesia associated with asymptomatic CT, but displaying new atypical genotypes.

2. Material & methods

2.1. Toxoplasma gondii isolates

T. gondii isolates were collected from ascitic fluid samples of two mothers living in French Polynesia who became infected during pregnancy. Informed consent was obtained from both mothers.
Table 1
Genotyping of T. gondii DNA with 15 microsatellite markers from amniotic fluid samples of the present cases (TgH13126 and TgH13119) and from 7 reference strains collected in America, Africa, Asia, and Europe.

<table>
<thead>
<tr>
<th>Type</th>
<th>Isolate</th>
<th>Origin</th>
<th>Host</th>
<th>microsatellite markers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atypical</td>
<td>TgH13126</td>
<td>French Polynesia</td>
<td>Human</td>
<td>TUR2: 291 242 203 160 336 167 274 358 213 190 145 115 269 89 304</td>
</tr>
<tr>
<td>Atypical</td>
<td>TgH13119</td>
<td>French Polynesia</td>
<td>Human</td>
<td>TUR2: 291 242 203 160 336 167 274 358 213 190 145 115 267 89 304</td>
</tr>
<tr>
<td>I</td>
<td>CT1</td>
<td>USA</td>
<td>Cow</td>
<td>TUR2: 291 248 209 160 342 169 274 358 209 168 145 119 265 87 306</td>
</tr>
<tr>
<td>II</td>
<td>TgH32006</td>
<td>France</td>
<td>Human</td>
<td>TUR2: 289 242 207 158 336 169 274 356 215 174 142 111 281 91 310</td>
</tr>
<tr>
<td>II</td>
<td>TgA32132</td>
<td>France</td>
<td>Sheep</td>
<td>TUR2: 289 242 207 158 336 169 274 356 221 174 138 111 277 91 312</td>
</tr>
<tr>
<td>III</td>
<td>NED</td>
<td>France</td>
<td>Human</td>
<td>TUR2: 289 242 205 160 336 165 278 356 209 190 147 111 267 91 312</td>
</tr>
<tr>
<td>Amazonian</td>
<td>VAND</td>
<td>French Guiana</td>
<td>Human</td>
<td>TUR2: 291 242 203 162 344 167 276 356 217 170 142 113 277 91 308</td>
</tr>
<tr>
<td>African I</td>
<td>TgH32052</td>
<td>Benin</td>
<td>Human</td>
<td>TUR2: 291 248 205 160 342 165 274 354 231 166 147 111 273 89 306</td>
</tr>
<tr>
<td>Chinese I</td>
<td>TgCIPRC04</td>
<td>China</td>
<td>Cat</td>
<td>TUR2: 293 242 211 160 336 169 274 354 215 172 145 123 281 93 308</td>
</tr>
</tbody>
</table>

Colored boxes indicate that alleles are specific for the three major clonal lineages: red for type I, green for type II, and blue for type III. Black boxes indicate that alleles are characteristic of atypical genotypes.

a TgA32132 is also known as FR-OVI-ARI061 strain; all reference strains, except TgCIPRC04, are available at the Toxoplasma Biological Resource Center (http://www.toxocrb.com).

The first mother, a 30-year-old woman living on Moorea Island (part of the Society Islands) had a Toxoplasma primary infection around the ninth week of amenorrhea (WA). The monthly serologic testing of seronegative pregnant women recommended by the French health authorities before the twelfth WA, allowed dating of the start of infection. In utero Toxoplasma infection was documented with a positive real-time polymerase chain reaction (PCR) targeting the RE-529 sequence of T. gondii DNA in an amniotic fluid sample drawn at WA 27 (Delhaes et al., 2013). CT was confirmed by the positive mice inoculation of the sample (Robert-Gangneux et al., 1999) and the spiramycin treatment (3,000,000 UIX3/d) started at WA 21 was changed to one combining pyrimethamine (50 mg/d) and sulfadiazine (1 gX3/d) with folinic acid supplementation until the delivery at WA 40. The fetal ultrasonography follow-up found no morphologic abnormalities, as did the neonatal transfontanellar ultrasonography, postnatal clinical and ophthalmological examinations. The newly born infant was treated with pyrimethamine (1.25 mg/kg/10 d) and sulfadoxine (25 mg/kg/10 d) complemented with folinic acid supplementation for 12 months. At month 17, the infant was still asymptomatic.

The second mother, a 27-year-old woman living on Raiatea Island (part of the Society Islands), had a primary infection around WA 28. In utero Toxoplasma infection was documented with a positive real-time PCR in an amniotic fluid sample drawn at WA 36. Consequently, spiramycin treatment that was started a few days before was changed to combining pyrimethamine and sulfadiazine with folinic acid supplementation until the delivery at WA 37. The fetal ultrasonography follow-up found no morphologic abnormalities. A cranial X-Ray of the infant showed four small punctiform frontal-parietal hyperdensities at Day 3, but the transfontanellar ultrasonography and the clinical and ophthalmological examinations were normal. The infant was treated by combining pyrimethamine and sulfadoxine with folinic acid supplementation for 12 months. At month 12, the infant was still asymptomatic. No mice inoculation of clinical samples was performed.

The maintenance and care of experimental animals comply with the requirements in animal experimentation in France.

2.2. Genotyping of Toxoplasma gondii DNA samples

The isolate collected in ascitic fluid samples of mice inoculated with the amniotic fluid of the first mother and the amniotic fluid DNA extract of the second mother were sent to the French National Reference Center for toxoplasmosis in Limoges, France. The genotyping analyses were performed with 15 T. gondii microsatellite loci in a single multiplex PCR assay on DNA extracted from the first isolate (designated as TgH13126) and directly on the amniotic fluid DNA extract containing the second isolate (designated as TgH13119) (Ajzenberg et al., 2010).

3. Results & discussion

The genotypes of the two T. gondii isolates were highly similar, since only one microsatellite marker was able to differentiate them, and unknown in the Toxoplasma database of the French National Reference Center. They were atypical and divergent from the common strains isolated in Europe, North America, South America, Africa and China (Table 1). To our knowledge, this is the first isolation and genotyping of T. gondii strains from French Polynesia. This is an overseas region of the French Republic, an archipelago of many volcanic islands located in the South Pacific Ocean; the most famous and populated island being Tahiti in the Society Islands group. The isolates were associated with a new and atypical genotype. This result supports the notion that genetic divergence of T. gondii could be related to the geographic origin of the strains (Ajzenberg et al., 2009; Elbez-Rubinstein et al., 2009). The two isolates were from Moorea and Raiatea, these islands are separated by 185 km. Raiatea would have been the first island colonized by the Polynesians and also the starting point for wide-ranging migrations to Hawaii, the Cook Islands and New Zealand. The climate in French Polynesia is tropical, oceanic and humid, but rendered temperate by the trade
winds. The maternal infections were acquired in mid-May 2011 in Moorea and in early July 2011 in Raiatea, both during the dry season, or austral winter. The close temporal-spatial distances could explain the high similarity found between the two genotypes.

These new atypical genotypes are associated with asymptomatic CT in humans after infection in the first and the third trimester of pregnancy. Recent reports suggested a higher virulence of some atypical strains than type II strains in CT (Delhaes et al., 2010; McLeod et al., 2012). Given the high genetic heterogeneity among atypical strains, it is also very likely that some of them may be less virulent, as suggested in our case reports. The first French Polynesian isolate was virulent for mice that died three weeks after IP inoculation and necropsy showed ascites and disseminated infections. *T. gondii* tachyzoites were microscopically observed in the ascites, brain, liver and spleen. This isolate is stored at the *Toxoplasma* Biological Resource Center, Limoges, France. It could be that this genotype is more adapted to humans than to mice. Nevertheless, we could not exclude that the parasiticidal treatment given to the fetus then to the newborns avoided severe toxoplasmosis. These genotypes might be common in humans living in this region. Factors that might explain the severity of toxoplasmosis seem to be complex. The parasite genotype may play a role, but it seems that the lack of adaptive host response is more probably associated with parasite virulence (Ajzenberg et al., 2009; Carme et al., 2009; Demar et al., 2007; Elbez-Rubinstein et al., 2009). Data on *T. gondii* genetic diversity are mainly available from French Guiana in South America (another tropical overseas region of France). There, highly divergent genotypes have been identified and some of them are associated with severe toxoplasmosis in immunocompetent patients. These virulent strains emerged from the forest-based cycle involving wild felids and their prey, and so are poorly adapted to humans (Demar et al., 2007). This *T. gondii* wildlife cycle coexists with a domestic cycle, in which cats play a central role. Both cycles can merge with the anthropization of the Amazonian forest (Mercier et al., 2011).

Few data are available about the epidemiology of toxoplasmosis in French Polynesia. Wallace reported a *Toxoplasma* dye-test antibody prevalence ranging from 29% to 71% in the adult population of Polynesia; the highest prevalence being in Tahiti (Wallace, 1976). The presence of the parasite in Pacific Islands is closely related to the presence of cats (Wallace, 1973, 1969). More data are available for the others Polynesian islands, particularly in Hawaii, where fatal toxoplasmosis (cerebral, myocardial and disseminated infections) have been reported in free-ranging and endangered crows, or ‘Alalā’ (Corvus hawaiiensis) (Work et al., 2000). Susceptibility to *T. gondii* might have been increased in released crows, as they were stressed, or lost weight during the reintroduction process. On Hawaii, *T. gondii* seems common, since 37% of feral cats have been found seropositive (Danner et al., 2007). In humans, toxoplasmosis has been reported as a major cause of chorioretinitis (83%) in patients from Rarotonga in Cook Islands (Heriot et al., 1982). As a consequence, additional identifications of *T. gondii* genotypes from cats, other animals and humans are needed in French Polynesia or Polynesia to determine the frequency and repartition of atypical genotypes and study their relation to virulence.

4. Conclusions

We described two new *T. gondii* genotypes from French Polynesia. These atypical strains are associated with asymptomatic CT in prenatally treated infants. Further work is needed to better understand the genetic diversity and the pathogenicity of *T. gondii* strains from this area.

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References


