ORIGINAL ARTICLE

Diversity and evolution of methods and practices for the molecular diagnosis of congenital toxoplasmosis in France: a 4-year survey

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Abstract

The prenatal diagnosis of congenital toxoplasmosis is currently based upon molecular biology using a sample of amniotic fluid. The vast majority of centres globally (and all centres in France) performing this diagnosis use 'in house' or laboratory-developed PCR assays. This may be the source of considerable inter-laboratory variation in the performances of the assays, hampering any valuable comparison of data among different centres. The present study was based upon questionnaires that were sent to 21–25 centres between 2002 and 2005 enquiring about methods and practices of the PCR-based prenatal diagnosis of congenital toxoplasmosis. An extreme diversity of PCR methods and practices was observed. Thus, in 2005, 35 PCR methods, differing in one of the main steps of the whole process, were reported as being in use for routine diagnosis, with nine centres using two or three methods. We provide comprehensive information on the extraction methods, DNA targets, primer pairs and detection methods used for this diagnosis, as well as their evolution, during the period of study. Interestingly, in this period (2002–2005), a rapid progression of the number of laboratories using real-time PCR technology, which increased from four to 19, was observed. We also studied general PCR practices concerning, for example, the number of reaction tubes used for each biological sample and the inclusion of controls. The return of information in a yearly report provided the opportunity for writing proposals aiming to improve laboratory practices for this diagnosis at the national level. The high diversity of methods and practices currently used emphasizes the need for external quality assessment of the performances of the molecular diagnostic methods.

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Introduction

Toxoplasmosis is an endemic protozoan disease whose prime public health importance is the result of possible vertical transmission from an infected mother to her foetus during pregnancy. Prenatal diagnosis (PND) of congenital toxoplasmosis (CT), wherever it has been implemented, has considerably improved the prognosis and outcome of infected children. Prevention of CT, including PND, has become a national policy in France ever since 1978 [1]. This national policy requires: (i) the detection and follow-up of non-immunized women as soon as possible during pregnancy with a series of serological tests; (ii) appropriate counselling aiming at limiting the risks of contamination; (iii) the detection and treatment of toxoplasmosis as early as possible aiming to prevent or limit transmission to the foetus and its consequences; (iv) PND of CT associated with monthly ultrasound examinations in case of a seroconversion; (v) combined

sulfadiazine-pyrimethamine treatment during pregnancy if CT is detected; and (vi) clinical, radiological and serological surveillance of neonates and infants at risk. This prevention programme is justified by the high prevalence of acquired toxoplasmosis in adults in France (approximately 44%) [2] and by the estimated yearly incidence of contamination in women during pregnancy (six or seven per 1000) and of congenital toxoplasmosis (approximately 0.1% of births) [3]. The programme was recently reinforced by the creation of a National Reference Centre for Toxoplasmosis (http://www.chu-reims.fr/professionnels/cnr-toxoplasmose-1/) which includes a 'pole' of molecular biology whose objectives include the improvement and standardization of the molecular diagnosis of CT, and whose coordinator is one of us (PB).

Indeed, molecular diagnostic tests, based upon PCR using amniotic fluid, have become essential in the diagnosis of CT; they have in great part superseded more classical methods, and have also led to the elimination of the need for cordocentesis [4]. In France, the PND of CT is made essentially in university hospitals, as well as in two large private biological diagnosis centres. Not all university hospital centres perform such testing because the centres and practitioners concerned need official authorization from the national health authorities to estabish this diagnosis, which is granted for 5 years.

However, despite their wide use, all PCR assays used for this application are still 'in-house'- or laboratory-developed methods (i.e. they have been set up independently in each laboratory using different targets and customized primers and reaction conditions). In addition, 'in-house' methods can largely differ at any step during the diagnostic process, such as the extraction method, the number of PCR tubes used for diagnosis, the inclusion of an internal control for the detection of inhibitors of the reaction, etc. These differences may be a source of considerable inter-laboratory variation in the performances of the assays, influencing the quality of the diagnosis and hampering any valuable comparison of data among centres. Previous studies have highlighted the lack of homogeneity and performance in European countries and underlined the need for guidelines [5,6]. In view of this heterogeneity, standardization of PCR methods and practices has become a strong desire for both health authorities and the community of clinical microbiologists. Such a standardization should in turn lead to improvement of the diagnosis of CT at a more global level, particularly regarding sensitivity, because parasite loads in this affliction are often very low [7].

To implement the harmonization of PND of CT in France, an early initiative for quality assurance in the molecular PND of toxoplasmosis was launched by the French association of hospital practitioners and teachers in Parasitology-Mycology (ANOFEL) in 2002. Briefly, a panel of *Toxoplasma gondii*-positive and -negative amniotic fluid samples prepared in Montpellier was sent blinded to participating centres for PCR testing on a yearly basis, allowing each centre to assess and follow its own performances in the molecular detection of CT [8].

A national survey was conducted in parallel from 2002 to 2005 aiming to assess the diversity and evolution of methods and practices used in this molecular diagnosis in France. The survey focused exclusively on the molecular PND of CT. The analysis of the data reported here provides an almost comprehensive description of these activities in France during the study period. It revealed a surprisingly high degree of diversity and the absence of any spontaneous trend toward standardization. Also, a massive introduction of quantitative 'real-time' PCR (qrtPCR) technology was observed during the study period, as opposed to 'conventional' PCR (cnPCR), a term used here for any form of end-point detection.

Materials and Methods

All laboratories of Parasitology–Mycology belonging to university hospitals, as well as one of the two officially authorized private diagnosis centres, were informed of the yearly external quality assessment (EQA) described previously [8]. Participating laboratories were free to enroll, anonymity of results was guaranteed, and no fees were imposed for participation. A questionnaire was sent every year to each participant, together with the EQA panel.

Participation in the EQA was anonymized through the use of letter codes and double-blinded cross-reading between the laboratories in Montpellier and Nice. An analysis of the questionnaires was performed after transcription of the data into spreadsheet software. The questionnaires included 11 queries concerning what we considered to be the most critical points of the PCR process. The query items are described below in the Results section. All answers to queries had to be given considering the routine practice of PND of CT, and not the procedures that could have been performed for the EQA only.

Results and Discussion

General observations

Between 2002 and 2005, the number of centres participating in the PND of CT increased from 21 to 25. All participants were from French University hospitals; none of the two private centres accredited for this diagnosis was involved. Most participants (23/25, i.e. 92% in 2005) were officially authorized