Genetic and virulence characterization of *Toxoplasma gondii* strains isolated from pigeons in Lisbon region

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INTRODUCTION

*Toxoplasma gondii* is an apicomplexan obligate intracellular parasite and the most extensively studied of the tissue-encysting coccidia. It has been estimated that one third of the world population has been infected (Mujie 2005, Sia 2005, Varela 2005). In most human adults it does not cause serious illness, however blindness and mental retardation can be caused in congenitally infected children and severe diseases in those with compromised immunity (Jubb 1983, Kirkpatrick 2003). A recent study indicated that infection with *T. gondii* is also associated with abdominal hernia (Samojlov 2005) and could increase the risk of brain cancer because it is a long-lived parasite that resides in the brain, where it provokes inflammation and inhibits apoptosis (Samojlov 2011). *T. gondii* has been isolated in three major groups, type I, II and III (Lafferty 2004). In pigeons, different prevalences have been described, ranging from 4.6% to 100% (Coelho 1976, Kirkpatrick 1984, Muir 2008, Varela 2008, Varela 2010, Yac 2013). In Portugal there is only a preliminary study in pigeons typing developed by a work group (de Nazare 2009). The microsatellite typing revealed that 75% of strains belonged to type II, 16.7% were type III and 8.3% was type I (de Nazare 2010). This work is part of a global study which aim is to help the understanding of the portuguese reality that concerns *Toxoplasma gondii* typing and virulence strains.

RESULTS

The isolation rate in mice of the 41 brain tissue was 58.5% (24/41). The 24 mice inoculated had antibodies (abs) Anti- *T. gondii* at 31st day after inoculation with one exception, which showed positive serology within 30 days after inoculation. None of the isolates were virulent to the mouse. In all mice with positive serology for *T. gondii* we performed a "post-mortem" microscopic analysis of the organs (brain, liver, lungs, heart and spleen), in which all had a normal conformation. We also performed a microscopic analysis where we observed brain cysts. *T. gondii* DNA was extracted (when present) from brain homogenates of the 41 pigeons and held the B1 gene PCR for the parasite identification. We obtained amplification in 29 of the 41 pigeons brains, which showed a *T. gondii* identifying rate of 71%. The remaining 12 samples were negative and was not observed any inhibition. Of the 29 positive samples to the B1 sequence gene, the genotyping by *Sac2* gene was achieved in 22 samples, for one of the three genotypes; 19 samples belong to type II, 2 to type II and 1 to type I. In other 5 samples we can't amplify one of the two ends of *Sac2* gene revealing the other end of *Sac2* gene that strains belonging to type I or to type II. The 2 remaining samples were not possible to genotype, possibly due to low concentrations of DNA. Unlike the amplified B1 gene sequence, the sequences of *Sac2* gene are single copy gene and the fragment is more difficult to amplify possible due to the DNA concentration or from the organic matrix of the initial product. However, it was possible genotype 22 samples, by these techniques. They were not amplified as a 5 multiplex PCR, but 3 multiplex PCR and the other 2 microsatellites were amplified separately. Genotyping by microsatellites showed concordance with previous results of the genotyping of the two ends of *Sac2* gene. The differentiation of strains was also performed in the brains of positive inoculated mice, being consistent with differentiation performed directly from the biological product (brain pigeon). Nevertheless, the differentiation by *Sac2* gene of these products revealed easier to perform than using the primary organic pigeon products, probably because the inoculation in mice enhances the strain concentration.

DISCUSSION AND CONCLUSIONS

The pigeons share the habitat with cats and humans, bands are observed in recreational areas such as urban parks, playgrounds and parks. The interaction between cats, birds and human population is quite evident favoring the local transmission of *T. gondii* between the definitive host and intermediate hosts, in the urban cycle of the parasite. The results of the inoculation in vitro of the brain homogenates showed pigeon isolation rates (58.5%) significantly higher when compared with previous studies, including the preliminary study in 2006 that the isolation rate in mice was 39.1% (de Nazare 2006) and another that was not achieved any isolation in mice (Samojlov 2005). The genotypic analysis revealed a majority of strains of type II, which is consistent with what has been described in Portugal, the rest of Europe and the USA (Quayle 2006, Quayle 2008, Honore 2008, Varela 1992, Varela 1997, Varela 2008). We also isolated strains of type III and type I. The identification of type III strains in animals has been reported by other authors, but the type I have been rarely found in animals has not been previously described in Portugal except in a preliminary study of our team at the 2008 (de Nazare 2008). The type I strains are usually associated with high virulence in laboratory mice, leading to death within days. This strain was identified by molecular biology and has not been isolated in vivo. The difficulty in isolation of strain may be related to the small number of cysts of the type I strains can develop, these type strains are consideredfee virulent. Genetic characterization of strains of *T. gondii* is far from its terminus, more sequences of different genes should be studied to help the understanding of the molecular epidemiology and genetic characterization of *T. gondii*, a relevant parasite for which these data are lacking. The combination of data from humans and animals, through the use of high resolution genetic characterization should improve our perception of *T. gondii*, which will be ultimately beneficial for the control of *T. gondii* transmission.

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Fig 1 – Works diagram