B1 (B2-B23) and TOX4-TOX5 genetic markers for molecular detection of *Toxoplasma gondii* in organic vegetables in the South of Brazil.

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Toxoplasmosis is a disease caused by *Toxoplasma gondii*, the most widespread protozoan parasite between human and animal populations. The severe forms of this disease can be observed in immunocompromised individuals and newborns with congenital infection, causing neuropsychomotor and ocular sequelae. *T. gondii* oocysts have high dispersion in the environment, it is an important source of infection and its detection is a great challenge. Thus, the aim of this study was to perform a molecular research of *T. gondii* in organic vegetables consumed in Maringá, Paraná, and to confirm the positive samples by genetic sequencing. A total of 77 samples of vegetables from street fairs were obtained in Maringá-PR and 50 g of each sample was taken for analysis in the Environmental Parasitology and Food Laboratory of the State University of Maringá. The DNA extraction was performed using a commercial kit and followed by Polymerase Chain Reaction (PCR) with two primers: B1 (B22-B23) and TOX4-TOX5, amplifying sequences of 115 and 529 bp, respectively, and viewed in 4.5% polyacrylamide. With B1 primer, two samples were positive, one chicory and one rocket. TOX4-TOX5 had one chicory positive. The genetic sequencing was performed in the positive sample which used TOX4-TOX5 primers, and presented 100% of similarity with a *T. gondii* sample, according to the BLAST software. However, due to the low genetic material concentration detected and the possibility of impurities that might interfere in the genetic sequencing, it was not possible to perform an analysis. The molecular analysis presents challenges that may hinder detection such as low amount of oocysts and humic acid presence, which consists polyphenols as interfering agents. Furthermore, previous researchers in this laboratory found out that the success of detection was only reached after inoculating ten oocysts in strawberries and crisp head lettuce. New techniques should be tested to make it possible to determine its genotypes at low DNA concentrations (LASS *et al.*, 2012). Finally, it would be a very important tool to detect this protozoan because of high genetic variability in South America, which contributes to increase the number of severe cases of chorioretinitis caused by toxoplasmosis (GRIGG *et al.*, 2015).

GRIGG, M. E.; DUBEY, J. P.; NUSSENBLATT, R. B. Ocular Toxoplasmosis: Lessons From Brazil. **American Journal of Ophthalmology**, v. 159, n. 6, p. 999–1001, 2015.

LASS, A. et al. The first detection of *Toxoplasma gondii* DNA in environmental fruits and vegetables samples. **European Journal of Clinical Microbiology & Infectious Diseases**, v. 31, p. 1101–1108, 2012.