# Supplementary Information

**First molecular detection of *Toxoplasma gondii* in vegetable samples in China using qualitative, quantitative real-time PCR and multilocus genotyping.**

Anna Lass[1,2*], Liqing Ma[2], Ioannis Kontogeorgos[2,3] Xueyong Zhang[2], Xiuping Li[2], Panagiotis Karanis[2,4 ,5]

[1]Department of Tropical Parasitology, Institute of Maritime and Tropical Medicine in Gdynia, Medical University of Gdansk, 9b Powstania Styczniowego Str., 81-519 Gdynia, Poland anna.lass@gumed.edu.pl

[2]State Key Laboratory of Plateau Ecology and Agriculture, Center for Biomedicine and Infectious Disease, Qinghai University, 1#Wei'er Road, Qinghai Biological Scientific Estate Garden, Xining 810016 - P. R. China, maliq67@hotmail.com, zhang_xyong@163.com, 2216659903@QQ.com

[3]Marine Sciences Department, School of Environment, University of the Aegean, University Hill, 88 100 Mytilene, Greece, ikontogeorgos@isc.tuc.gr

[4]Cologne University, Medical Faculty and University Hospital, Cologne, Germany, panagiotis.karanis@uk-koeln.de

[5]University of Nicosia, Medical School, 46 Makedonitissas Avenue, CY-2417 P.O. Box 24005, CY-1700, Nicosia, Cyprus

*Corresponding author: Anna Lass, Department of Tropical Parasitology, Institute of Maritime and Tropical Medicine in Gdynia, Medical University of Gdansk, 9b Powstania Styczniowego Str., 81-519 Gdynia, Poland anna.lass@gumed.edu.pl

**Supplementary Table S1.** Characteristics of standard curve generated by amplification of the serial dilutions of standard DNA.

| Standard template No | NS | Cq(ΔR) Set1 | Cq(ΔR) Set2 | Cq(ΔR) Set3 | Cq(ΔR) av. | Cq(ΔR) SD |
|---|---|---|---|---|---|---|
| **1** | 1.00e+008 | 10.02 | 10.15 | 10.20 | 10.13 | 0.09 |
| **2** | 1.00e+007 | 13.50 | 13.58 | 13.42 | 13.50 | 0.08 |
| **3** | 1.00e+006 | 17.18 | 17.39 | 17.34 | 17.30 | 0.11 |
| **4** | 1.00e+005 | 20.44 | 20.45 | 20.72 | 20.54 | 0.16 |
| **5** | 1.00e+004 | 23.99 | 24.32 | 24.42 | 24.24 | 0.23 |
| **6** | 1.00e+003 | 27.60 | 27.38 | 26.96 | 27.31 | 0.33 |
| **7** | 1.00e+002 | 30.15 | 30.84 | 30.80 | 30.60 | 0.39 |
| **8** | 1.00e+001 | 32.32 | 33.17 | 35.66 | 33.72 | 1.74 |
| **9** | 1.00e+000 | 39.38 | 34.87 | – | 37.12 | 3.19 |
| **Characteristics of the standard curve (produced by Agilent Aria Software v1.3)** | | | | | | |
| **E [%] =** 98**, $R^2$ =** 0.99**, S =** -3.37**, $y_{int}$ =** 37.32 | | | | | | |

NS – initial concentration of standard DNA [copies/µL]
Cq (ΔR) set1-3 – Cq values obtained for particular set of replicates
Cq (ΔR) av. – average Cq values obtained from three sets of replicates
Cq (ΔR) SD – standard deviation obtained from three sets of replicates
E – the amplification efficiency
$R^2$ – the correlation coefficient
S – slope of the standard curve
$y_{int}$ – the y-intercept for log(N) = 0

**Supplementary Table S2.** Summary result of *T. gondii* qPCR performed for vegetable samples collected from open markets in the Xining City, Qinghai Province, P.R. China.

| Positive sample No | Cq(ΔR) 1 | Cq(ΔR) 2 | Cq(ΔR) 3 | Cq(ΔR) av. | Cq(ΔR) SD | NPav. | OCSav. | SCSav. | CCSav. |
|---|---|---|---|---|---|---|---|---|---|
| **32** | 31.55 | 31.68 | 31.60 | 31.61 | 0.06 | 4.94e+001 | 1.7 | 3.4 | 13.6 |
| **50** | 27.90 | 27.72 | 27.59 | 27.74 | 0.15 | 6.99e+002 | 25 | 50 | 200 |
| **61** | 17.50 | 17.41 | 17.50 | 17.47 | 0.05 | 7.74e+005 | 27640 | 55280 | 221120 |
| **140** | 20.59 | 20.47 | 20.53 | 20.53 | 0.06 | 9.56e+004 | 340 | 683 | 2730 |
| **192** | 34.73 | 34.17 | 33.4 | 34.10 | 0.66 | 9.66e+000 | 0.3 | 0.69 | 2.76 |
| **203** | 27.67 | 27.78 | 27.74 | 27.73 | 0.06 | 7.00e+002 | 25 | 50 | 200 |
| **212** | 17.41 | 17.47 | 17.65 | 17.51 | 0.12 | 7.56e+005 | 27000 | 54000 | 432000 |
| **229** | 31.21 | 30.80 | 31.65 | 31.22 | 0.42 | 6.64e+001 | 2.4 | 4.8 | 19.2 |
| **254** | 30.55 | 30.44 | 29.90 | 30.23 | 0.29 | 1.29e+002 | 4.6 | 9.2 | 36.8 |
| **265** | 33.93 | – | 38.43 | 36.18 | 3.18 | 5.30e+000 | 0.18 | 0.36 | 1.44 |

Cq (ΔR) 1-3 – Cq values obtained for positive vegetable samples in qPCR run in triplicate together with standard curve
Cq (ΔR) av. – average Cq values obtained from triplicates of positive vegetable samples
Cq (ΔR) SD – standard deviation obtained from triplicates of positive vegetable samples
NP – initial copy number (number of copies of *T. gondii* B1 gene) present in positive vegetable samples calculated based on standard curve
OCS – calculated equivalent of initial number of *T. gondii* oocysts present in positive vegetable samples
SCS – calculated equivalent of initial number of *T. gondii* sporocysts present in positive vegetable samples (OCS x 2, one oocyst consist of two sporocysts)
CCS – calculated equivalent of initial number of *T. gondii* cells (sporozoites) present in positive vegetable samples (OCS x 8, one oocysts consist of two sporocysts filled with four sporozoites each)

**Supplementary Table S3.** Results of inhibition test performed for *Toxoplasma gondii* positive vegetable samples collected from open markets located in Xining City, Qinghai Province, P.R. China.

| Number of template | Cq (ΔR) for IPC |
|---|---|
| **32** | 15.12 |
| **50** | 15.37 |
| **61** | 15.15 |
| **140** | 15.32 |
| **192** | 15.09 |
| **203** | 15.58 |
| **212** | 15.69 |
| **229** | 15.32 |
| **254** | 15.39 |
| **265** | 15.26 |

IPC – internal positive control
Cq (ΔR) IPC - Cq values obtained after amplification of internal positive control (IPC)

## Supplementary data for internal positive control (IPC) used in the study

DNA extracted from laboratory strain of *Acanthamoeba* spp. cultivated axenically in the laboratory of the Department of Tropical Parasitology, Medical University of Gdansk was used to construct IPC. Briefly, a 180 bp fragment of 18SrRNA gene of *Acanthamoeba* spp. was amplified using primers AcantF900 and AcantR1100 [1] and cloned into a plasmid. Detection of IPC was performed with real-time PCR using TaqMan probe labelled with 5' Cy5/3'BHQ3 fluorophores under amplification conditions described in the manuscript.

## Bibliography

1. Qvarnstrom, Y., Visvesvara, G. S., Sriram, R. & Silva, A. J. da. Multiplex Real-Time PCR assay for simultaneous detection of *Acanthamoeba* spp., *Balamuthia mandrillaris*, and *Naegleria fowleri*. *Journal of Clinical Microbiology* **44**, 3589–3595 (2006).