Occurrence of *Toxoplasma gondii* in raw meat products in Poland. Molecular characteristics and viability assessment of isolated parasites

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Raw meat and raw meat products are considered as the main sources of *Toxoplasma gondii* infection for humans in Europe, however data in this issue for Poland are still insufficient. The aim of the study was to determine the prevalence of *T. gondii* in raw meat products sold in selected regions of Poland, on the basis of DNA detection, as well as molecular characteristics and assessment of the viability of isolated parasites.

In total, 871 samples of raw smoked meat products, sausage, ham and minced meat, collected in the following regions of Poland: Małopolskie, Podkarpackie, Lubelskie, Podlaskie, Warmińsko-Mazurskie and Pomorskie (2019–2020), were tested.

Samples were digested by pepsin solution, followed by the DNA isolation (QIAGEN). Detection of *T. gondii* DNA was performed by nested and real time PCR (B1 gene fragment). In order to determine the clonal type of the isolated parasite, RFLP-PCR was performed using the 12 genetic markers: SAG1, SAG2 (5'and 3'), altSAG2, SAG3, GRA6, BTUB, C22-8, C29-2, L358, PK1 and APICO, according to the method by Su et al. (2010). After digestion with restriction enzymes, the amplification products were identified on an agarose gel under ultraviolet light. The amplicons were also sequenced and analyzed with NCBI database. Cell culture and bioassay on mice were performed to assess the viability of isolated *T. gondii*.

In total, 54 of 871 tested samples (6.2%) were PCR positive. The highest percentages of positive results were found for samples from Pomorskie (12%) and Warmińsko-Mazurskie (10%), lowest from Podlaskie (0.5%) and Lubelskie (2%) regions. The percentages of positive results for particular types of meat products ranged from 1.5% (smoked meat products) to 9% (sausages). RFLP-PCR and sequence analysis showed mainly *T. gondii* clonal type II (46%), followed by type III (34%). Combinations of various clonal types of *T. gondii* were also found (20%). Viable *T. gondii* was isolated from 17 (2%) samples of meat products.

The detection of *T. gondii* DNA and a live parasite in 6.2% and 2% of the tested samples of raw meat products, respectively, indicates a real risk to the health of consumers.