

Molecular detection of *Toxoplasma gondii* in natural surface water bodies in Poland

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ABSTRACT

The aim of this study was molecular detection of *Toxoplasma gondii* in 36 natural surface water bodies in Poland, including preliminary genotype identification and determination of co-occurrence of this parasite with other protozoa that have been detected in previous studies. The examined DNA samples were obtained before to detect *Cryptosporidium*, *Giardia* and free-living amoebae. Nested polymerase chain reaction (PCR) based on B1 gene and sequencing was performed for both confirmation of parasite presence in water and genotype identification. *T. gondii* DNA was detected in 19.4% (7/36) water bodies, while in the case of other studies, *T. gondii* prevalence ranged between 0% and over 56%. These differences may be caused by natural variations in *T. gondii* occurrence as well as different sample volumes and methods of sample processing or DNA isolation and detection. Two cases of double contamination were reported: *T. gondii* with *Cryptosporidium parvum* and *T. gondii* with potentially pathogenic *Acanthamoeba* T4 genotype, thus there is a possibility of mixed infection in humans after occasional contact with water. Obtained *T. gondii* strains were genetically identical or closely similar (99.8%) to RH strain representing genotype I, however, further examinations involving more loci will be conducted to identify the genotype.

Key words | double contamination, genotype, natural water bodies, *Toxoplasma gondii*

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INTRODUCTION

Toxoplasma gondii is a parasitic protozoan infecting warm-blooded animals, including humans. Its definitive hosts are felids that excrete faeces containing unsporulated forms of *T. gondii* oocysts into the environment and sporozoites develop inside oocysts after a few days (Robert-Gangneux & Dardé 2012). Three stages are responsible for transmitting the infection: oocysts containing sporozoites (waterborne and foodborne transmission route), tissue cysts containing bradyzoites (foodborne transmission route) and tachyzoites (congenital infection or rare cases of foodborne infection) (Robert-Gangneux & Dardé 2012). Water contaminated with faeces containing oocysts is an important source of human infection either by direct consumption or by the use of contaminated water in food processing and

preparation (Robert-Gangneux & Dardé 2012). Cases of waterborne outbreaks of toxoplasmosis have been described since 1979 in Panama, Canada and Brazil and other outbreaks in some countries are supposed to be caused by consuming contaminated water (Jones & Dubey 2010).

There are a few studies describing methods that allow for recovery and detection of *T. gondii* oocysts from water, e.g., centrifugation or filtration, flocculation, sucrose flotation, immunomagnetic separation, detection by immunofluorescence microscopy, bioassays, cultured cells infection, lectin-magnetic separation (LMS) and molecular techniques as well as combinations of these methods (Jones & Dubey 2010; Harito *et al.* 2017). However, studies on *T. gondii* occurring in natural water bodies are not

numerous and have been performed in Poland, Germany, France, Bulgaria, Russia and Iran so far (Villena *et al.* 2004; Sotiriadou & Karanis 2008; Aubert & Villena 2009; Sroka *et al.* 2010; Gallas-Lindemann *et al.* 2013; Polus & Kocwa-Haluch 2014; Mahmoudi *et al.* 2015). In this study, DNA isolated earlier from samples obtained from 36 Polish natural water bodies was investigated, using polymerase chain reaction (PCR) product sequencing, to determine the frequency of *T. gondii* occurrence and preliminary genotype identification of this parasite in water environment. The aim of this study was also to reveal *T. gondii* co-occurrence with protozoa detected earlier in the water bodies (Adamska *et al.* 2014, 2015).

METHODS

In this study, the DNA samples obtained earlier from surface water bodies (Adamska *et al.* 2014, 2015) were examined to detect the presence of *T. gondii* DNA. The 50 L water samples were collected from 36 water bodies in north-western Poland, between winter 2009 and autumn 2012 (Adamska *et al.* 2014, 2015). The water bodies were chosen because of their vicinity to inhabited and/or recreational areas. In order to increase the number of protozoan (oo)cysts in the samples, they were processed earlier using a Manual Filta Max[®] Wash Station (Idexx Laboratories, USA) and auxiliary equipment according to the 1623 Method (Adamska *et al.* 2014, 2015). The method was modified, and immunomagnetic separation and immunofluorescence were not performed. After samples' filtration, (oo)cysts elution and concentration, a part of each obtained sample was used for amoebae culture (Adamska *et al.* 2014). Then, DNA was extracted from the cultures and amoebae DNA was detected from the obtained DNA samples (Adamska *et al.* 2014), as well as DNA was isolated directly from concentrated (oo)cysts mix and obtained DNA samples were examined earlier to detect the presence of *Cryptosporidium* and *Giardia* DNA (Adamska *et al.* 2015). The latter DNA samples were also used for *T. gondii* DNA detection in this study.

For molecular identification of *T. gondii*, nested PCR was performed in order to amplify a 531-bp region of tandemly arrayed 35-fold-repetitive B1 gene (Grigg &

Boothroyd 2001). All analyses were carried out in two replicates. DNA isolates for positive controls were obtained from tachyzoites originating from a culture of *T. gondii* ME49 strain (genotype II) thanks to the courtesy of Dr Jacek Sroka from the National Veterinary Research Institute, Pulawy. PCR products were visualized in 1.5% agarose gels stained with ethidium bromide.

Both strands of all obtained amplicons were sequenced (Macrogen, Korea) with the amplification primers and obtained sequences aligned with each other using ClustalW (Mega 5.10 software). They were also initially aligned with homology sequences published in GenBank using BLAST (www.ncbi.nlm.nih.gov) and then using Mega 6.06 software, with ClustalW (Tamura *et al.* 2013). The ends of the alignment were trimmed to form blunt ends on all the sequences in the alignment. The final alignment covered nucleotides corresponding to nucleotide positions 1 to 531 of *T. gondii* strains analysed in this study. Sequences analysis was used for both confirmation of *T. gondii*'s presence in water and preliminary genotype identification.

RESULTS AND DISCUSSION

The results of this study show the presence of *T. gondii* DNA in natural surface water bodies in north-western Poland. Among all examined water bodies, *T. gondii* DNA was detected in 19.4% (7/36) of them – four lakes (Glebokie, Miedwie, Miejskie and Jeleni Staw), two rivers (Plonia and Swina) and one pond (Brodowski). Sroka *et al.* (2010) detected *T. gondii* DNA in 10.5% of water bodies from eastern Poland, while Polus & Kocwa-Haluch (2014) did not detect the presence of *T. gondii* DNA in surface water samples collected in the area of Cracow. There are a few similar studies on *T. gondii* in natural surface water bodies in other countries. In France, Villena *et al.* (2004) detected *T. gondii* DNA in 8% of water samples of different origin, whereas a study by Aubert & Villena (2009) revealed the presence of *T. gondii* DNA in 5% of raw surface water samples. Sotiriadou & Karanis (2008) detected the presence of *T. gondii* DNA in 13.9% or 44% of natural water samples from Bulgaria and 12.5% or 56.25% from Russia, depending on the method used. Gallas-Lindemann *et al.* (2013) did not detect *T. gondii* DNA in samples from German surface

water bodies. Mahmoudi *et al.* (2015) detected *T. gondii* DNA in 9.1% of Iranian natural water bodies. The differences between the prevalence of *T. gondii* DNA obtained in this study and in studies of other authors may be caused by many factors. One of the reasons may be natural variations in *T. gondii* occurrence connected with different environmental conditions that are important for oocysts' survival and the structure of hosts' populations. Both intermediate and definitive hosts are necessary in the *T. gondii* life cycle and their abundance and distribution in the environment may also have an influence on the prevalence of *T. gondii*. Other reasons for the differences may be the different number of water bodies examined or water samples' volumes. The differences may also be caused by various water filtration methods and protocols of DNA isolation and detection, including both detection method and applied molecular assay, therefore the results are difficult to compare.

The sequences analysed in this study have been deposited in GenBank under accession numbers KT266790–KT266796. Four variants of the 531-bp sequence of the *T. gondii* B1 gene were obtained. Four sequences (from strains TG18 – Miejskie Lake, TG34 – Swina River, TG38 – Brodowski Pond and TG42 – Miedwie Lake) were identical to each other, while the rest of them (strains TG1 – Glebokie Lake, TG40 – Jeleni Staw Lake and TG41 – Plonia River) were unique. On the basis of molecular studies, *T. gondii* strains have been divided into three major genotypes (I, II and III) and a number of atypical ones (Robert-Gangneux & Dardé 2012). The alignment of obtained sequence variants revealed that the four identical strains are identical to homologous sequence of *T. gondii* RH strain (AF179871) representing genotype I. Similarity of the remaining three unique variants to AF179871 amounts to 99.8%. Among the three predominant clonal lineages, the difference at DNA sequence level is up to 2% (Robert-Gangneux & Dardé 2012), so the strains described in this study seem to represent genotype I. Data on genotypes of other *T. gondii* strains detected in natural water bodies and the surrounding environment, which is one of the sources of oocysts in water, are not numerous. Based on one locus RFLP analysis, two strains, representing genotype I and II/III, were found in water bodies from eastern Poland (Sroka *et al.* 2010), and predominance of genotype

I in soil and plants in northern Poland was demonstrated by Lass *et al.* (2009, 2012). However, genotype I is particularly found in Europe (Robert-Gangneux & Dardé 2012), while genotype II predominates and genetic typing based on one locus is not enough to detect all atypical genotypes that may occur (Ajzenberg *et al.* 2002). Additional examinations, involving more loci, will be conducted in further studies to identify the genetic structure of the strains from the water environments described in this paper.

The presence of *T. gondii* strains in water bodies examined in this study may pose a threat for people as all of them are used as bathing places and occasional water consumption may take place during bathing. Moreover, Miedwie Lake serves as a drinking water source. There are a few cases of *T. gondii* oocysts occurring in public drinking water or infections caused by consuming drinking water containing oocysts (Aubert & Villena 2009; Jones & Dubey 2010). Oocysts can remain viable for long periods of time in water and can resist chemical and physical treatments applied in water treatment plants (Robert-Gangneux & Dardé 2012). Therefore, water treatment may not be fully effective and the presence of viable oocysts in drinking water from Miedwie is possible. However, detected *T. gondii* DNA may originate not only from oocysts, but also from disintegrated tissues of infected animals as IMS was not performed after water sample processing. Regarding Glebokie Lake, two cases of double contamination with *T. gondii* and a protozoan detected earlier (Adamska *et al.* 2014, 2015) were revealed; with potentially pathogenic *Acanthamoeba* T4 genotype (winter 2009) and with *C. parvum* (autumn 2010). Thus, there is a possibility of mixed infection in humans after contact with the lake water.

CONCLUSIONS

Studies on occurrence of *T. gondii* in natural water bodies are not numerous. The prevalence of this parasite in different countries has a wide range and may depend on many factors, e.g., natural variations in *T. gondii* occurrence, sample volumes and methods of sample processing or DNA isolation and detection. The presence of *T. gondii* strains in the water bodies examined in this study may

pose a threat for people after occasional water consumption including mixed infection.

ACKNOWLEDGEMENTS

I thank Dr Jacek Sroka from National Veterinary Research Institute, Pulawy for giving DNA isolates of *Toxoplasma gondii* for positive controls. This study was partly supported by the Ministry of Science and Higher Education, grant no. N N404 248635.

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First received 12 September 2017; accepted in revised form 28 April 2018. Available online 17 May 2018