Echinococcus multilocularis in water supplies of endemic areas in Poland and China

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INTRODUCTION. Water, among other risk factors, has been indicated as one of the possible routes of transmission of parasites to humans. *Echinococcus multilocularis* is a parasite causing a dangerous zoonosis, alveolar echinococcosis (AE), number of which is increasing worldwide. This tapeworm is widely distributed throughout the Northern Hemisphere. The estimated global human burden of AE is 666,434 DALYs, with 91% of cases recorded in China (Torgerson *et al.*, 2010). Humans become infected through the ingestion of *E. multilocularis* eggs excreted with the faeces of definitive hosts. The data on the distribution of *E. multilocularis* eggs in the environment is very limited. Studies concerning contamination of soil, plants and mushrooms has been confirmed i.a. in Japan (Matsudo *et al.*, 2003), Poland (Szostakowska *et al.*, 2014; Lass *et al.*, 2015), Switzerland (Federer *et al.*, 2016), and France (Umhang *et al.*, 2017). Water route of transmission still remains unchecked.

AIM OF THE STUDY. The current research was undertaken in two endemic echinococcosis areas: the Qinghai province, China and Warmia-Masuria province, Poland. The main aim of the projects was to determine the occurrence of *E. multilocularis* in different types of water supplies, including wastewater and surface water, as well to compare usefulness of different molecular detection methods in screening environment for *E. multilocularis*.

MATERIAL AND METHODS. In total, 259 water samples were collected, including 222 samples from wastewater treatment plants (WWTP's), a slaughterhouse, a cattle farm and rivers in the Qinghai province, Western China, and 37 samples from lakes and surface wells in the Warmia-Masuria province, Poland. The material was filtered using either 293-mm membrane filters (1.2 μ m, Millipore) in case of Chinese samples or membrane capsule filters (1 μ m, Envirochek) in case of Polish samples and subsequently analysed with chosen molecular detection methods: nested PCR, real-time PCR, and LAMP. Positive samples were additionally sequenced in order to confirm if obtained sequences belong to *E. multilocularis*.

RESULTS. Of the samples examined, E. multilocularis DNA was found in 13 (5,8%) of the samples collected in China, and in 2 (5,4%) of the samples collected in Poland. Sequencing of positive samples confirmed that the PCR products were fragments of the E. multilocularis mitochondrial 12S rRNA gene. All molecular assays used showed high effectiveness in the detection of the parasite's DNA in water, exciding 90%. We confirmed presence of the tapeworm DNA in wastewater (WWTPs and slaughterhouse) and water from a cattle farm in China, and in lakes from

endemic communities in Poland. The results indicate that water should be considered a source of $\it E. multilocularis$ infections in humans and animals in endemic areas and more studies are needed in this field.

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