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Bioaccumulation of human waterborne protozoa by zebra mussel (*Dreissena polymorpha*): Interest for water biomonitoring

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ABSTRACT

Cryptosporidium parvum, *Giardia duodenalis* and *Toxoplasma gondii* are ubiquitous pathogens, which waterborne transmission has been largely demonstrated. Since they can be found in various watercourses, interactions with aquatic organisms are possible. Protozoan detection for watercourses biomonitoring is currently based on large water filtration. The zebra mussel, *Dreissena polymorpha*, is a choice biological model in ecotoxicological studies which are already in use to detect chemical contaminations in watercourses. In the present study, the zebra mussel was tested as a new tool for detecting water contamination by protozoa. *In vivo* exposures were conducted in laboratory experiments. Zebra mussel was exposed to various protozoan concentrations for one week. Detection of protozoa was realized by Taqman real time qPCR. Our experiments evidenced *C. parvum*, *G. duodenalis* and *T. gondii* oocyst bioaccumulation by mussels proportionally to ambient contamination, and significant *T. gondii* prevalence was observed in muscle tissue. To our knowledge, this is the first study that demonstrates *T. gondii* oocyst accumulation by zebra mussel. The results from this study highlight the capacity of zebra mussels to reveal ambient biological contamination, and thus to be used as a new effective tool in sanitary biomonitoring of water bodies.

1. Introduction

Cryptosporidium spp and *Giardia* spp are the main protozoa associated with waterborne outbreaks (Fayer et al., 2004; Baldursson and Karanis, 2011) where ninety percent are associated with water (Guy et al., 2003). In Europe, an epidemiological study reported by the ECDC (European Centre for Disease prevention and Control, 2011) underline an increase of Cryptosporidiosis cases from 2006 to 2009, with 8016 cases in 2009. A higher rate in the 0- to 4-year-old followed by the 5- to 14-year-old children was noted. Giardiasis cases have been stable over the last four years, with a total of 93,375 cases in 2009. Nonetheless, authors also emphasize the lack of reported cases in some countries, which does not allow for reliable results. In the USA, cryptosporidiosis and giardiasis are responsible cause over 2700 and 3500 hospital

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registrations per year and costs of $45 million and $35 million, respectively (Collier et al., 2012). *Toxoplasma gondii* infection is a common parasitic human infection although an efficient immune system keeps the parasite from causing illness. Toxoplasmosis is associated with two modes of transmission: a horizontal one *via* zoonoses, whereby foodborne parasites could represent 50% of transmission, and a vertical one from mother to foetus (Scallan et al., 2011). Infection could be due to an environmental protozoan form, the *oocysts*, or to a tissue form, the *cysts*. A recent study underlines that oocyst infection is an important and unrecognised source of congenital toxoplasmosis and leads to higher incidence and severity of ocular toxoplasmosis rather than tissue cyst infection (Boyer et al., 2011). Various meaningful outbreaks around the world are associated with *T. gondii* in water. In the USA, waterborne protozoa like *Toxoplasma* are responsible for almost 9000 cases per year at a direct total healthcare cost of $398 million (Karanis et al., 2007; Collier et al., 2012).

Water protozoan detection is presently based on the filtration of large amounts of water. Karanis et al. report a recent overview of water analysis towards *Toxoplasma*. They listed several methods from sample filtration to molecular tools and bioassays (Karanis et al., 2013). These methods were optimized for water monitoring, which is a relatively pure matrix. Thus, results are not only based on protozoan prevalence in samples but also on the methods chosen to detect protozoa. However, protozoan paucity in results does not necessarily mean protozoan paucity in watercourses. Whatever the analytical methods chosen, water analysis is based on large quantities of water, is expensive, inaccurate and time-consuming (Toze, 1999). In watercourses, protozoa are subject to water characteristics that affect (oo)cyst transport dynamics. Indeed, dilution events and physicochemical parameters could influence spatial and temporal pathogen distribution. Shapiro et al. (2012) recently highlighted that *T. gondii* oocysts and surrogate attachment was greater in marine and estuarine waters, mainly due to salinity which alters the surface charge of *T. gondii* oocysts. Aggregation results in bigger particles likely to be more available to filter-feeder organisms such as aquatic invertebrates.

Depending on chemical contaminants, various biomonitoring programs use aquatic invertebrates to reveal water contamination (US Mussel Watch: Golberg, 1986; The French Réseau d'Observation de la Contamination CHimique du littoral: http://envlit.ifremer.fr/surveillance; Besse et al., 2013). Organisms such as filter-feeders could accumulate and concentrate contaminants in high quantities directly in their tissues. Ecotoxicological studies have already used aquatic invertebrates such as the zebra mussel, *Dreissena polymorpha*, as sentinel species for the quality of freshwater ecosystems. In fact, zebra mussel is easy to collect in large numbers and to maintain in laboratory. They are sedentary and have a high filtration rate that allows for the bioaccumulation of both organic and inorganic contaminants in defined sites (Bervoets et al., 2005). In order to evidence new contamination types, a new method like bioaccumulation by invertebrates could be used as a tool for protozoan biomonitoring in watercourses. Since aquatic invertebrates are already routinely used in biomonitoring programs, they could also reflect biological contamination levels. *Cryptosporidium parvum* and *Giardia duodenalis* bioaccumulation by zebra mussel has already been demonstrated in previous studies (Lucy et al., 2008). There are no data in the literature about *T. gondii* bioaccumulation by zebra mussel, but bioaccumulation by the marine mussel *Mytilus galloprovincialis* (Arkush et al., 2003) and the oyster *Crassostrea virginica* (Lindsay et al., 2004) has been demonstrated.

The aim of this study is twofold: First, we attempted to underline the capacity of zebra mussel to bioaccumulate the three protozoa in a simultaneous *in vivo* exposure to 560 (oo) cysts of each pathogen in laboratory conditions. Thus, *T. gondii* accumulation was compared with the other two pathogens since *C. parvum* and *G. duodenalis* are known to be accumulated by zebra mussel. Second, single *in vivo* exposures were conducted to refine the previous results, using two pathogen concentrations: an environmental concentration of 20 (oo) cysts per mussel and per day (Graczyk et al., 2004) and a tenfold higher concentration of 200 (oo)cysts per mussel and per day to point out a dose–response relationship of the three pathogens. Then, using the pathogen that has been accumulated in the highest proportion, organotropism was studied. Muscle as a hemolymph reservoir, gills which are the first structures in contact with the environment, and the digestive gland which is the major site of xenobiotic uptake, were analysed at various sampling times to understand how protozoa transit inside mussels.

2. Materials and methods

2.1. *Exposure protocol*

Purified *C. parvum* oocysts and *G. duodenalis* cysts preparations were purchased from Waterborne™, Inc. (New Orleans, LA, USA) and kept at 4 °C until use. *T. gondii* oocysts were generously gifted by J.P. Dubey (USDA, Beltsville, USA). The oocysts were obtained from the feces of cats experimentally infected by strain ME49 genotype II. They were stored at 4 °C in an aqueous solution containing 2% H_2SO_4 until use.

Zebra mussel between 20 and 25 mm long were collected at Commercy (Northeastern France) along the Meuse east channel (N48°45′26.13″,E5°36′14.51″). They were acclimated in tanks for two weeks before exposure and analyses to ensure that they were protozoan-free on the sampling day and on the first exposure day (D0). The tanks were filled with Cristaline Aurèle (Jandun, France), drinking water at a constant temperature of 14 ± 1 °C and with controlled aeration (Conductivity, 427 ± 46 µS/cm; Nitrates, 10 ± 3.5 mg/L; Nitrites, 0 mg/L; Ammonium, 0 mg/L). The mussels were fed *ad libitum* twice a week with a mixture of two species of microalgae, *Chlorella pyrenoidosa* and *Scenedesmus obliquus*.

In a first set of experiments, mussels were randomly divided into 2 groups of 35 specimens each. Each group was placed in a 5 L tank that contained 3 L of spring water. For 7 days, the mussels were exposed to 560 (oo)cysts of each protozoan per mussel and per day in simultaneous exposure in order to compare the respective accumulation rates of the three pathogens. In a second set of experiments, mussels were randomly divided into 14 groups of 50 specimens each. Each group was placed in a 15 L tank that contained 4.5 L of