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First report of Tunisian coastal water contamination by protozoan parasites using mollusk bivalves as biological indicators

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ABSTRACT

In order to establish seawater contamination by emerging protozoan parasites, we used *q*PCR to molecularly characterize and evaluate the parasitic burden of *Giardia duodenalis*, *Cryptosporidium* spp., *Toxoplasma gondii*, and *Cyclospora cayetanensis* in 1255 wild bivalve mollusks collected along the Tunisian coasts. *T. gondii*, *G. duodenalis* and *C. cayetanensis* were detected in 6.9% (99% CI = 1.6–12.2%) pools of *Ruditapes decussatus*. None of the samples were found positive to *Cryptosporidium* spp.; 6.6% pools of *R. decussatus* were positive for *T. gondii* Type I, 1.6% for *G. duodenalis* assemblage A, and 1.6% for the association *T. gondii* Type I/*C. cayetanensis*/*G. duodenalis* assemblage A. *R. decussatus* harbored up to 77500 oocysts/sample of *T. gondii*, up to 395 cysts/sample of *G. duodenalis*, and 526 oocysts/sample of *C. cayetanensis*. These results provide the first evidence that the Tunisian coasts are contaminated by zoonotic protozoan parasites that can constitute a direct or indirect risk for human health.

1. Introduction

Worldwide, authorities responsible for protecting the natural environment and human health are increasingly interested in seawater quality. The ecological pressure caused by intense anthropogenic activities means that all the world's marine environments, including shallow coastal areas, river estuaries and water basins, are considered a kind of "*litmus test*" because they may be contaminated by runoff from agricultural, suburban and urban land and by wastewater discharges. Coastal ecosystems are threatened by the improper disposal of sewage, inefficient/non-functioning treatment plants, illegal wastewater discharge, artificial and natural runoff, and a wide range of industrial waste products (Clark, 2001; Barhoumi et al., 2014). Feces from humans, their pets, domesticated and wild animals may carry numerous pathogenic microorganisms, which can easily contaminate the sea and its inhabitants when released into estuaries and marine environments worldwide (Adell et al., 2014; Giangaspero et al., 2014).

Able to filter large volumes of water and to retain and concentrate microorganisms, shellfish are considered the best biological indicators of the health conditions of marine environments (Chaffai, 2014; Zuykov et al., 2013) They are used extensively worldwide to monitor pollution in aquatic environments, thereby saving time, labor, and money, in comparison with water analysis (Palos Ladeiro et al., 2014).

Shellfish can accumulate several pathogens of anthroponotic and/or zoonotic origin, and those which cause most concern are viruses (e.g., Norovirus, Hepatitis A virus) and bacteria (e.g., pathogenic *Escherichia coli*, *Campylobacter jejuni*, *Salmonella* spp., *Vibrio vulnificus*, *Vibrio cholerae*, *Vibrio parahaemolyticus*), but also protozoan parasites (including *Cryptosporidium*, *Cyclospora*, *Giardia* and *Toxoplasma*). Detection of these pathogens in shellfish indicates fecal contamination of seawater, provides a good indication of the biological pollution of marine ecosystems, and thus identifies possible risks for human health.

Worldwide, *Cryptosporidium* spp., *Giardia duodenalis*, *Cyclospora cayetanensis* and *Toxoplasma gondii* have been detected in farmed or wild shellfish in lagoons and other marine environments (reviewed by Robertson, 2007). Along the Mediterranean Sea coasts, several edible and inedible shellfish have been found to carry protozoan pathogens, either alone and/or in association. These include *Cryptosporidium* spp. in Spain, Portugal and Italy (Giangaspero et al., 2005, 2014; Gomez-Couso et al., 2006; Melo et al., 2006); *G. duodenalis* in Spain and Italy (Gómez-Couso et al., 2005; Giangaspero et al., 2014), *Toxoplasma* in Turkey and Italy (Putignani et al., 2011; Aksoy et al., 2014), and *Cyclospora* in Turkey (Aksoy et al., 2014).

Tunisia's coastline is 1300 km long; the northern coast is fringed by short floodplains with lagoons and coastal lakes, whereas the southern

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coast is low and has large bays into which several rivers and watercourses flow. Fishing activities, aquaculture and shellfish farms are very widespread along the Tunisian coast and in saltwater catchment areas (FAO, 2015).

The presence of enteric viruses in Tunisia has been documented in sewage samples and in shellfish tissues (Elamri et al., 2006; Sdiri-Loulizi et al., 2010); whereas the presence of protozoan parasites has been recorded in sludge and wastewater samples (Ben Ayed et al., 2009, 2012).

These studies highlight the need to protect public health, and suggest that seawater may be subject to fecal contamination. However, no research has yet investigated biological contamination with pathogenic protozoa along the Tunisian coasts. In this context, we aimed to provide a first evaluation of fecal pollution of Tunisian coastal waters via molecular detection and characterization of some important intestinal protozoan parasites (i.e., *G. duodenalis*, *Cryptosporidium* spp., *C. cayetanensis* and *T. gondii*), using wild shellfish found along the Tunisian coastline as environmental biosentinels.

2. Materials and methods

2.1. Study area

Four Tunisian coastal areas were investigated, from North to South: Bizerte Lagoon, Monastir Bay, Chebba and the Gulf of Gabès (Fig. 1).

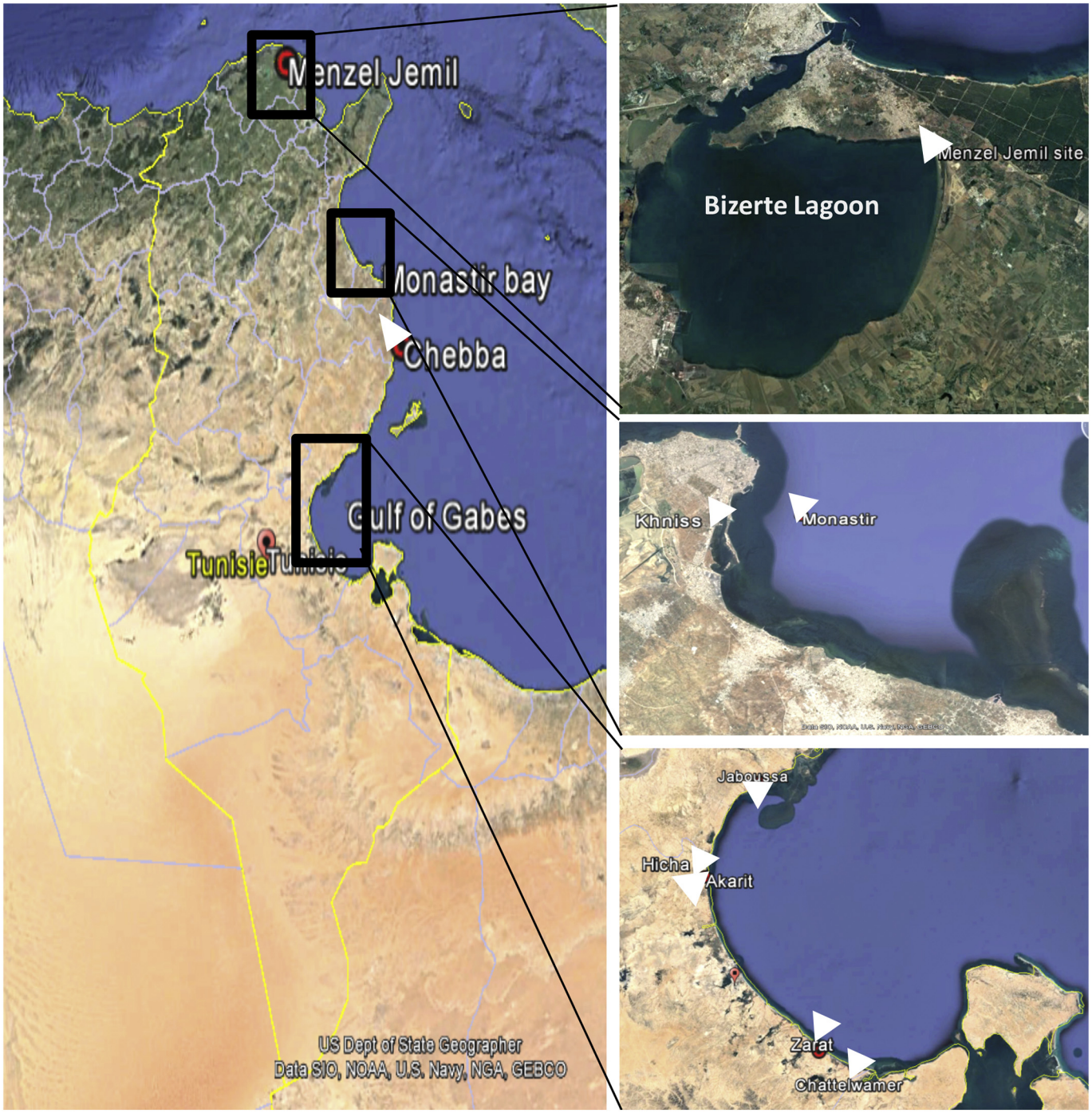

Fig. 1. Map of collection sites on Tunisia coasts. Sites are indicated with white triangles.

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