Enzyme immunoassay use in the identification of *Giardia* spp. in *Perna perna* mussels destined for human consumption*

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ABSTRACT. do Couto M.C.M., Silva V.L., Pinheiro J. & do Bomfim T.C.B**. Enzyme immunoassay use in the identification of *Giardia* spp. in *Perna perna* mussels destined for human consumption.** [Utilização do ensaio imunoenzimático no diagnóstico de *Giardia* spp. em moluscos *Perna perna* destinados ao consumo humano.] *Revista Brasileira de Medicina Veterinária, 38(supl. 3):165-170, 2016.* Programa de Pós-Graduação em Ciências Veterinárias, Anexo 1, Instituto de Veterinária, Universidade Federal Rural do Rio de Janeiro, BR 465 - Km 7, Seropédica, RJ 23890-000, Brasil. E-mail: melcouto@ufrrj.br

Marine bivalve molluscs are important due to the expansion of mariculture and because they are considered bioindicators of environmental pollution. Bivalve molluscs are capable of filtering large volumes of water and can accumulate waterborne pathogens, such as cysts of *Giardia intestinalis*, especially in their gills and digestive gland. Thus, the ingestion of raw or undercooked molluscs can be a potential source of human infection. This study aimed to use the enzyme-linked immunosorbent assay (ELISA) technique to detect cysts of *Giardia* spp. in tissues of mussels of the species *Perna perna* destined for human consumption in the coast at the Municipality of Mangaratiba in the State of Rio de Janeiro, Brazil. Each sample was prepared from a pool of 10 animals, totalling 72 samples of mussel tissue that were evaluated for the presence of *Giardia* spp. by ELISA. For sampling, only individuals with an average of 6 cm of valve length were analyzed, which is considered the ideal size for consumption. In each sample, the individuals were dissected, and only the gills and digestive gland were used, which were homogenized with the aid of a mixer and filtered to remove coarse residues. The use of the enzyme kit followed the recommendations of the manufacturer with minor modifications. Among the 72 samples used, only 22% were positive for the presence of *Giardia* spp. antigens. The obtained results were evaluated by colorimetry and by an ELISA plate reader with a 450/630 nm filter. Based on the results, the authors suggest that the use of the immunoassay kit is effective in the diagnosis of *Giardia* spp. and could be considered a screening method prior to analysis by other diagnostic methods.

KEY WORDS. Marine bivalve mollusc, ELISA, bioindicator, environmental pollution.

RESUMO. Moluscos bivalves marinhos apresentam importância devido à expansão da maricultura e por serem considerados bioindicadores de poluição ambiental. São capazes de filtrar grandes

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volumes de água, podendo acumular patógenos de veiculação hídrica, como cistos de *Giardia intestinalis*, principalmente em brânquias e glândula digestiva. Dessa forma, a ingestão de moluscos crus ou mal cozidos pode ser uma fonte potencial de infecção humana. O presente estudo teve por objetivo a utilização da técnica de imunodiagnóstico (ELISA) para a detecção de cistos de *Giardia* spp. em tecidos de mexilhões da espécie *Perna perna* destinados para consumo humano, no litoral do Município de Mangaratiba, Rio de Janeiro, Brasil. Cada amostra foi preparada a partir de um "pool" de 10 animais, totalizando 72 amostras de tecidos de mexilhões *Perna perna* que foram avaliadas para a presença de *Giardia* spp. através do teste de ELISA. Para a amostragem foram utilizados somente indivíduos com média de 6 cm de comprimento de valva, tamanho considerado ideal para o consumo. Em cada amostra, os indivíduos foram dissecados sendo utilizado apenas brânquias e glândula digestiva, que foram homogeneizados com o auxílio de um mixer e filtradas para retirada de resíduos grosseiros. A utilização do kit enzimático seguiu as recomendações do fabricante com pequenas modificações. Dentre as 72 amostras utilizadas apenas 22% apresentaram-se positivas para a presença de antígenos de *Giardia* spp. Os resultados obtidos foram avaliados através de colorimetria e por um leitor de placa de ELISA com filtro de 450/630 nm. Com base nos resultados obtidos os autores sugerem que a utilização do kit imunoenzimático é eficiente no diagnóstico da *Giardia* spp., podendo ser considerada como uma metodologia de triagem para posteriormente serem analisadas por outros métodos de diagnóstico.

PALAVRAS-CHAVE. Molusco, *Perna perna*, ELISA, *Giardia*, bioindicador.

INTRODUCTION

Coastal areas are home to numerous animal species that represent an important source of food for humankind. Among the methods for marine resource development and extraction, mariculture deserves special mention.

Brazil has enormous potential for the cultivation of shellfish, offering a coastal area of approximately 8,000 km that is favourable for mariculture (Pereira & Soares-Gomes 2002). Although the country has been experiencing increasing shellfish production, there are some problems that hinder the consolidation of Brazil's position as a world producer, including the deficit in hygiene and sanitation control of the animals and the areas where they are cultivated (Melo 2007).

Increasingly, coastal regions are subject to various environmental problems, such as high concentrations of domestic sewage released *in natura* into aquatic environments due to rampant urban growth and the tourism incentive in these regions (IBGE 2010). The increasing discharge of treated and/or untreated sewage into rivers and seas directly compromises the quality of these water resources because in addition to contributing to elevated levels of organic nutrients in these environments, such discharge can introduce a number of human enteric pathogens (Fayer et al. 2004).

Bivalve molluscs are easily adaptable to cultivation (Fernandes 1985) and are capable of withstanding large environmental variations (Andreu 1976). In recent decades, these animals have acquired great ecological importance because they can be used as biological indicators in the control of water quality (Widdows et al. 1995), mainly due to their ability to filter large quantities of water and retain particles and microorganisms in their tissues (Palos Ladeiro et al. 2013). Bivalve molluscs are widely recognized as bioindicators of aquatic heavy metal and pesticide contamination (O'connor 2002, Palos Ladeiro et al. 2013), in addition their use as tools to monitor the presence of faecal bacteria, viruses and parasites in these environments (Pommepuy et al. 2004). Thus, these animals are widely utilized as sentinel organisms in aquatic biomonitoring (Palos Ladeiro et al. 2013).

During the process of water filtration in search of food, molluscs such as oysters and mussels are able to concentrate infective forms of protozoa in their tissues, thus becoming an important source of infection for humans (Tamburrini & Pozio 1999, Robertson 2007, Giangaspero et al. 2009, Francavilla et al. 2012, Palos Ladeiro et al. 2013). The quality of molluscs used for human consumption is directly related to the environment where the molluscs are cultivated. There are several forms of contamination from the aquatic environment that directly affect mariculture, including animal faeces carried in water runoff and release of partially treated or *in natura* sewage into rivers, lakes, lagoons and seas.

Some studies have tried to elucidate the role of bivalve molluscs as transmitters of human protozoan infections (Gomez-Couso et al. 2005, Schets et al. 2007); these studies have mainly targeted mollusc species that are used commercially, such as oysters and clams (Palos Ladeiro et al. 2013). Among the diseases caused by protozoa that are transmitted by the consumption of raw or undercooked molluscs,

giardiasis is commonly diagnosed in humans and animals (Thompson 2004, Thompson & Monis 2004). The prevalence of giardiasis may vary from 0.8% to 7% in developed countries and from 20% to 40% in developing countries (Julio et al. 2012).

The genus *Giardia* has only a single species able to parasitize mammals, *Giardia intestinalis* (syn. *G. duodenalis* and *G. lamblia*), which is subdivided into eight distinct genotypes (A, B, C, D, E, F, G and H), each with the capacity to infect different groups of mammals. Among the varieties mentioned above, it is believed that only two (A and B) have zoonotic characteristics, i.e., the ability to parasitize humans (Monis et al. 2009, Robertson 2009, Feng & Xiao 2011).

Host infection by *Giardia* occurs through the ingestion of the infective form of the parasite (cysts) in water and/or contaminated foods, which are subsequently eliminated by the parasitized individual via the faeces. The symptomatology of giardiasis is variable; hosts may show clinical signs such as diarrhoea, abdominal pain, nausea and vomiting or may be asymptomatic (Mohammed Mahdy et al. 2008, Cotton et al. 2011, Palos Ladeiro et al. 2013). Malnutrition and severe weight loss are commonly reported in infected children and young animals (Silva et al. 2009, Geurden et al. 2010).

The present study aimed to evaluate the occurrence of *Giardia* in environmental samples obtained from *Perna perna* mussels by the detection of specific protozoan antigens (using ELISA) on the coast at the Municipality of Mangaratiba in the State of Rio de Janeiro, Brazil.

MATERIAL AND METHODS

Location, collection and processing of material

During a period of 12 months, six monthly samples (n = 72) of the mussels *Perna perna* were collected around Mangaratiba Bay in the Costa Verde of Rio de Janeiro state, Brazil, in areas where extractivism of this animal commonly occurs. The sampling was conducted using only adult individuals with the ideal size for slaughter, i.e., a valve length of 6 cm (Avelar 1998).

The collected molluscs were placed into chilled coolers until their arrival at the Laboratório de Protozoologia in the Departmento de Parasitologia Animal, IV, UFRRJ, where they were measured, cleaned and separated into groups. Each sample comprised 10 animals, totalling 60 individuals per collection. Each mollusc was removed from its valve and dissected to remove the gills and digestive glands, which were the only parts used in this study. The tissues were washed in PBS solution by horizontal agitation, macerated and homogenized in a processor. The resulting material was filtered using a drum sieve with gauze to retain the maximum amount of coarse residues and then centrifuged in 15 ml tubes. The obtained pellet was stored at a temperature of 4 °C for subsequent analysis of the presence of *Giardia* spp. antigens using a commercial immunodiagnostic *kit*.

Enzyme-linked immunosorbent assay diagnosis

The detection of *Giardia* spp. antigens in the *P. perna* molluscs was performed using the enzyme-linked immunosorbent assay *kit* (ELISA) *Giardia stool antigen detection* (© IVD Research, Inc., Carlsbad, CA, USA), following the manufacturer's instructions with minor modifications. Among these modifications, 50 µl of each sample was diluted in 150 µl of dilution buffer and homogenized with a pipette into 200 µl microtubes; 100 µl of the material previously diluted were added in the microwell test plate containing anti-*Giardia* antibodies used for the diagnosis.

The samples were tested by colorimetry and evaluation of optical density (OD) of the samples using an ELISA plate reader with 450/630 nm filter. The material evaluated by colorimetry was considered positive for the presence of *Giardia* spp. antigens when a yellow coloration was present, and an absorbance reading was considered to be positive when the OD value was greater than or equal to 0.08.

RESULTS

In the present study, a total of 72 samples of the *P. perna* mollusc were analyzed for the presence or absence of *Giardia* spp. antigens via an enzyme-linked immunosorbent assay. Of these samples, 22.2% (16/72) were positive for the presence of *Giardia* spp. antigens based on the colorimetry evaluation and on the absorbance reading using the ELISA plate reader (Table 1).

DISCUSSION

The present study evaluated the occurrence of *Giardia* spp. antigens in bivalve molluscs of the *P. perna* species in the region at the Mangaratiba Bay, on the coast of Rio de Janeiro state, Brazil.

Reports in the literature using ELISA for the diagnosis of *Giardia* spp. are scarce, and there have been no reports of the detection of protozoan antigens in marine molluscs. However, the use of the ELISA technique in the diagnosis of infection in humans has been successful, as reported by some authors (Julio et al. 2012, Christy et al. 2012, Den Hartog et al. 2013), in accordance with the results obtained in the present study where the presence of *Giardia* spp. antigens was diagnosed in macerated gills and digestive glands of bivalve molluscs of the species *P. perna*.

The detection techniques for the studied parasite are diverse, and immunofluorescence and poly-

Table 1. Occurrence of Giardia spp. in *Perna perna* mussels collected at Mangaratiba Bay in the State of Rio de Janeiro, Brazil, diagnosed by enzyme-linked immunosorbent assay *kit* (ELISA); OD – Optical density.

Collection	Samples	Colorimetry	OD	Collection	Samples	Colorimetry	OD
1	1	+	0.662	7	37	–	0.006
	2	+	0.403		38	–	0.004
	3	+	0.456		39	–	0.004
	4	–	0.002		40	–	0.007
	5	–	0.004		41	–	0.007
	6	–	0.006		42	+	1.225
2	7	–	0.007	8	43	+	1.262
	8	–	0.003		44	+	1.276
	9	–	0.006		45	+	1.232
	10	–	0.006		46	–	0.006
	11	–	0.004		47	–	0.007
	12	–	0.005		48	–	0.037
3	13	–	0.007	9	49	–	0.002
	14	–	0.028		50	–	0.002
	15	–	0.002		51	–	0.007
	16	–	0.006		52	+	0.505
	17	–	0.007		53	+	1.625
	18	–	0.004		54	+	0.304
4	19	–	0.004	10	55	–	0.005
	20	–	0.006		56	–	0.003
	21	–	0.006		57	–	0.005
	22	–	0.006		58	–	0.005
	23	–	0.003		59	–	0.004
	24	–	0.003		60	–	0.004
5	25	+	1.100	11	61	–	0.006
	26	+	1.055		62	–	0.004
	27	+	1.398		63	–	0.001
	28	–	0.001		64	–	0.007
	29	+	0.274		65	–	0.005
	30	+	0.661		66	–	0.004
6	31	–	0.003	12	67	–	0.003
	32	–	0.000		68	–	0.007
	33	–	0.002		69	+	0.744
	34	–	0.004		70	–	0.004
	35	–	0.003		71	–	0.006
	36	–	0.054		72	–	0.007

merase chain reaction (PCR) are the most frequently used (Palos Ladeiro et al. 2013). However, the present study demonstrated that ELISA is also an efficient technique for the detection of protozoan antigens; thus, the authors suggest it as an additional diagnostic method.

In a study conducted in Portugal, Julio et al. (2012) assessed the prevalence of *G. intestinalis* in children using two diagnostic techniques: microscopes and enzyme-linked immunosorbent assays. Both of the techniques were able to diagnose the presence of the parasite; however, ELISA was reported to be more sensitive of the two. This fact corroborates the previous discussion, demonstrating that enzyme immunoassays can be used to obtain a rapid and efficient diagnosis of the presence of *Giardia* spp.

In addition to the speed and efficiency of diagnosis due to the high sensitivity and specificity of ELISA, Den Hartog et al. (2013) mentions that ELISA is a potential tool in the diagnosis of this and other protozoan infections because it has a relatively low cost of acquisition, and the kit is easy to use and transport.

The high occurrence of *Giardia* spp. antigens observed in marine bivalves obtained in the studied region is troubling, demonstrating that the water quality where these animals were found was not suitable for recreational purposes and may be considered a risk to human and animal health. This concern for the environment and human health has also been discussed by Palos Ladeiro et al. (2013), who also emphasized the scarcity of studies investigating the problems of environmental contamination and protozoan infections that can affect humans.

The contamination of the aquatic environment is of concern mainly due to the ability of molluscs to retain *Giardia* spp. cysts during the process of filtering water in search of oxygen and nutrients.

These animals are most often eaten raw, usually at the very location where they are collected. This behaviour is considered highly dangerous because the mollusc can become a vehicle for ingesting the protozoan. This fact has been consistently described in the literature (Gomez-Couso et al. 2004, Graczyk et al. 2006, Robertson, 2007, Giangaspero et al. 2009, Francavilla et al. 2012, Palos Ladeiro et al. 2013), demonstrating great concern among researchers who use this information to attempt to alert the population about the risk of infection by *Giardia intestinalis* associated with the consumption of raw or undercooked shellfish.

CONCLUSIONS

Taking the findings of this study into consideration, the authors suggest that the enzyme-linked immunosorbent assay (ELISA) is a fast and effective method to diagnose *Giardia* spp. in bivalve molluscs, aiding in the monitoring of environmental contamination and in the control of infection in humans and/or other animals by this protozoon.

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