

Presence of *Blastocystis* spp. in the mollusc *Crassostrea virginica*, in Mexico City

Presencia de *Blastocystis* spp. en el molusco *Crassostrea virginica*, en la Ciudad de México



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Ignacio Martínez-Barbabosa¹, Elena Marcia Gutiérrez-Cárdenas^{1*}, Aída Hamdan-Partida¹, Jaime Bustos-Martínez¹, Michael Shea²

¹Departamento de Atención a la Salud. Área de Ciencias Básicas. Universidad Autónoma Metropolitana-Xochimilco. Calzada del Hueso 1100. Colonia Villa Quietud, Del. Coyoacán C.P. 04960 México D.F. México.

²Departamento de Educación y Comunicación. Universidad Autónoma Metropolitana- Xochimilco. Calzada del Hueso 1100. Colonia Villa Quietud, Coyoacán. C.P. 04960 México D.F. México.

ABSTRACT: The aim of this study was to identify, by microscopic analysis and polymerase chain reaction (PCR), the presence of *Blastocystis* spp. in the mollusc *Crassostrea virginica* sold in Mexico City. Thirty specimens of the mollusc were obtained. The intestine of each was dissected and *Blastocystis* spp. was isolated from the faeces for microscopic analysis. Of those samples positive to *Blastocystis*, 15 (50 %) were selected, the genomic DNA was extracted and the PCR amplification of the 26S rRNA gene was performed. Of all the samples, a fragment of 1 770 bp was amplified in 13 of them. These results show the presence of *Blastocystis* spp. in the oysters.

Key words: *Blastocystis* spp., *Crassostrea virginica*, oysters, PCR.

RESUMEN: El objetivo de este trabajo fue identificar, mediante análisis microscópico y reacción en cadena de la polimerasa (PCR), la presencia de *Blastocystis* spp. en el molusco *Crassostrea virginica* vendido en mercados de la Ciudad de México. Se obtuvieron 30 ejemplares del molusco. Se disecó el intestino de cada uno y se aisló *Blastocystis* spp. de las heces para análisis microscópico. De las muestras positivas a *Blastocystis* spp. se seleccionaron 15 (50 %), se les realizó la extracción del ADN genómico y la amplificación por PCR del gen ARNr 26S. Del total de muestras, en 13 se amplificó un fragmento de 1 770 pb. Estos resultados muestran la presencia de *Blastocystis* spp. en las ostras.

Palabras clave: *Blastocystis* spp., *Crassostrea virginica*, ostras, PCR.

*Autor para correspondencia: Elena Marcia Gutiérrez-Cárdenas. E-mail: emgcard@correo.xoc.uam.mx

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Blastocystis spp. specimens have been reported in various hosts such as rodents, birds, reptiles, snakes, insects, and fleas (1). It has not yet been determined if these carriers are involved in the transmission to humans (2). It seems that each host has a definite relationship with a particular subtype of the protozoa (3). Man is a host to this parasite and various subtypes can infect humans and animals (4). *Blastocystis* spp. may potentially be a zoonotic parasite (5). Reproduction is by binary fission, endodyogeny, schizogony, and plasmotomy. It has four forms: vacuolar, granular, amoeboid and cystic (6).

The prevalence of this parasite is increasing. In a parasitic transition analysis (1990-2010), *Blastocystis* is found to be the most important emerging protozoan (7). Transmission is faecal-oral, as with other intestinal protozoa. The encystment occurs in the intestine where the vacuolar form is released. This may become granular or amoeboid. It is evacuated as a cyst (8). It is uncertain whether eating uncooked seafood (molluscs), which is a possible carrier of *Blastocystis* spp., results in transmission or cross infection. However, the pathogenic mechanism of this enteric parasitic protists, if it exists, remains controversial. Nevertheless, clinicians diagnose and treat patients with the infection (9).

The aim of this study was to identify, by microscopic analysis and polymerase chain reaction (PCR), the presence of *Blastocystis* spp. in the bivalve mollusc.

Thirty oysters of the species *C. virginica* were collected from the main fish market in Mexico City. Samples were washed with individual brushes and sterile distilled water before being opened. Subsequently, each mollusc was placed individually in a sterile Petri dish to identify and dissect the intestine. The intestinal content of the molluscs was examined microscopically. First, there was an analysis by direct examination of the faeces, using lugol. A Carl Zeiss microscope at 40x objective and 100x oil immersion was also used for identification (10).

With the aim of accumulating mass to obtain genetic material, 15 faecal samples (from oysters), in which *Blastocystis* spp. had been identified, were resuspended in isotonic saline solution at a final volume of 500 µL. The samples were incubated in modified Boeck-

Drbohlay medium (MBDM) together with animal serum and Locke solution. A basic antibiotic solution was added, so that the concentration of the antibiotic was based on ampicillin (4 mg/mL), streptomycin (1.25 mg/mL) and amphotericin B (0.006 mg/mL). The mixture was left for 1 to 2 days. The cultures were incubated at 37°C in an anaerobic atmosphere. Being clearly identified, the cell culture of the parasite was centrifuged. The supernatant was decanted and the culture pellet was washed with PBS buffer. The biomass obtained was resuspended in an Eppendorf tube with 180 µL of PBS. Genomic DNA was extracted using the Guanidine thiocyanate method together with the chloroform isoamyl alcohol method (11).

AMPLIFICATION OF 26S RRNA GENE OF *BLASTOCYSTIS* SPP. BY PCR FROM SAMPLES OF OYSTER FAECES

Fifteen specimens were subjected to PCR analysis. The subunit 26S rRNA gene was amplified by PCR using specific primers to the parasite (12): BH1: 5' GCT TAT CTG GTT GAT CCT GCC AGT 3' and BH2: 5' TGA TCC TTC CGC AGG TTC ACC TAC A 3' under the following conditions: initial denaturation at 94°C for 5 min, 35 cycles at 94°C for 1 min, annealing at 55 °C for 1 min, 72°C for 1.5 min with a final extension at 72°C for 5 min. An amplicon of 1 770 pb was obtained. The positive control used in the reaction was obtained from a human infection. With the aim of confirming amplification, the results were observed in an agarose gel electrophoresis at 1.5 %. The gel was subsequently stained with ethidium bromide and visualized in the Gel Doc XR Bio-Rad transilluminator using Quantity One software.

The observation of the cystic form showed heavily stained granules in the periphery regions and spread out on the membrane. The central body (characteristic of *Blastocystis*) affects the surrounding cytoplasm. The peripheral granules can be clearly seen. A large central vacuole occupies much of the space of the cytoplasm (Fig. 1).

The results obtained by PCR (Fig. 2) showed the presence of *Blastocystis* spp. in *C. virginica* oysters. These samples had previously been found to be positive microscopically.

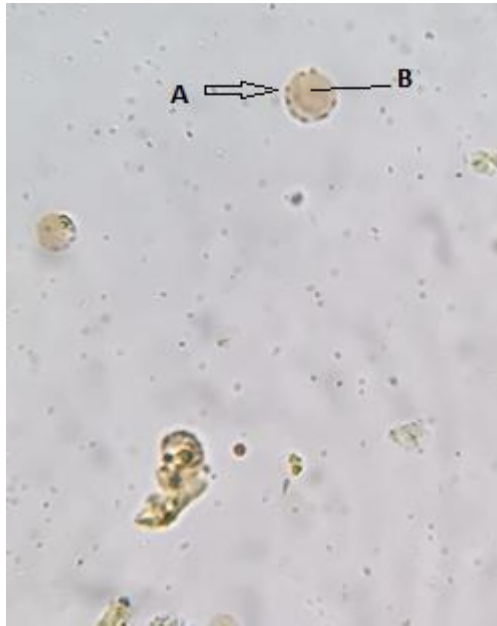


FIGURE 1. *Blastocystis* spp. cyst in *C. virginica* faeces with Lugol stain. A: peripheral granules. B: central body. (100X). / *Quiste de Blastocystis spp. de heces de C. Virginia con tinción de Lugol. A: gránulos periféricos. B: Cuerpo Central (100X).*

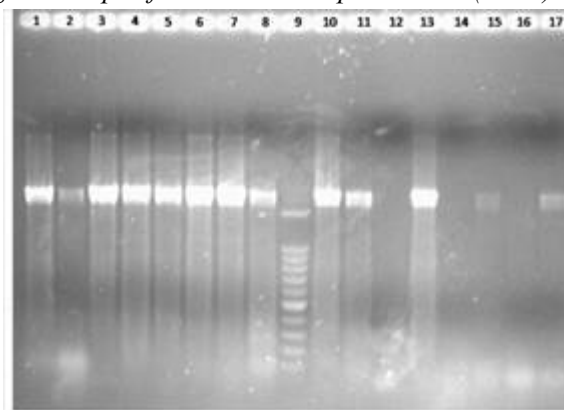


FIGURE 2. Amplification of the 26S rRNA gene of *Blastocystis* spp. by PCR from oyster faeces. Agarose gel at 1.5 %. Lanes 1-8 and 10-15 samples; lane 9: molecular weight marker (100pb); lane 16: negative control; lane 17: positive control. / *Amplificación del gen 26S rRNA de Blastocystis spp. por PCR a partir de muestras de heces de ostra. Gel de agarosa al 1,5 %. Líneas 1-8 y 10-15 muestras; línea 9: marcador de peso molecular del carril 9 (100 pb); línea 16: control negativo; línea 17: control positivo.*

Mexico ranks fourth in mollusc production in Latin America (13). The Gulf of Mexico is the source of 90 % of the national production. Much of this production is exported and this is what gives the epidemiological importance to the findings of this study. The consumption of raw oysters involves a potential risk of zoonotic infection with *Blastocystis* spp. (14). Studies suggest an association between this disease and intestinal protozoa (15).

This research confirmed the presence of this parasite in oysters, microscopically and by PCR. The infection degree found was 50 % using the

microscope. The 43 % (13/30) of the samples were found to be positive by observation and they were analyzed using PCR.

The morphological similarities between different subtypes suggest that the combination of microscopic and PCR analyses may be the solution to find a definitive confirmation of a given subspecies (16). There is information on *Blastocystis* spp. structure and taxonomy; however, many other areas are still rather obscure. Above all, its pathogenicity needs to be further researched (17). A study such as this one needs to be supplemented by the use of more

advanced molecular typification and larger samples. Studies suggest that the human gut microbiota has a molecular level influence on the parasitic pathogenicity (18).

This research provides evidence of the existence of *Blastocystis* spp. in *C. Virginica* oysters using microscopic and PCR analysis.

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The authors of this work declare no conflict of interest.

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