



EPIZOOTIOLOGY OF *Perkinsus* sp. INFESTING *Crassostrea rhizophorae* FROM THE SEMI-ARID REGION, BRAZIL

EPIZOOTIOLOGIA DE Perkinsus sp. INFESTANDO Crassostrea rhizophorae DA REGIÃO SEMI-ÁRIDA, BRASIL

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Abstract

The coast of the semi-arid region of Brazil is known for the presence of large estuaries inhabited by numerous species of edible bivalve mollusks. The oyster *C. rhizophorae* is naturally found in estuarine environments along the Brazilian coast, fixed on substrates, mainly in the roots of mangroves. Protozoa of the genus *Perkinsus* are important parasites of marine bivalves, sometimes causing significant pathological damage throughout the world. This study reports the occurrence of parasites *Perkinsus* sp. in mangrove oysters from Porto de Mangue estuary (Rio Grande do Norte state) and Icapuí estuary (Ceará state), two estuaries of the semi-arid region of Brazil. In Porto do Mangue estuary, oysters were collected in December 2016, January and February 2017. In Icapuí estuary, oyster sampling occurred in September, October and November 2017. Two fragments of the gills were incubated in Ray's Fluid Thioglycollate Medium – RFTM. The ecological and parasitological parameters were calculated. Five of the ninety oysters collected in Porto do Mangue estuary were parasitized, which corresponded to a mean prevalence of 5.6%, and in Icapuí estuary 18 of the 120 oysters collected were parasitized, which corresponded to a mean prevalence of 15.0%. The intensity of infection by *Perkinsus* sp. ranged from very mild to mild.

Keywords: mangroves; oyster; perkinsiosis; RFTM.

Resumo

A costa da região semi-árida do Brasil é conhecida pela presença de grandes estuários habitados por numerosas espécies de moluscos bivalves comestíveis. A ostra *Crassostrea rhizophorae* é encontrada naturalmente em ambientes estuarinos ao longo da costa brasileira, fixados em substratos, principalmente nas raízes dos manguezais. Os protozoários do gênero *Perkinsus* são parasitos importantes de bivalves marinhos, às vezes causando danos patológicos significativos em todo o mundo. Este estudo relata a ocorrência de parasitos *Perkinsus* sp. nas ostras de mangue do estuário de Porto do Mangue (estado do Rio Grande do Norte) e estuário de Icapuí (estado do Ceara), duas regiões do semiárido do Brasil. No estuário de Porto do Mangue as amostragens de ostras ocorreram nos meses de dezembro de 2016, janeiro e fevereiro de 2017. No estuário de Icapuí as amostragens de ostras ocorreram nos meses de setembro, outubro e novembro de 2017. Dois fragmentos branquiais foram incubados em Ray's Fluid Thioglycollate Medium (RFTM). Os parâmetros ecológicos e

parasitários foram calculados. Cinco das noventas ostras coletadas no estuário de Porto do Mangue estavam parasitadas, que correspondeu a uma prevalência média de 5,6%. No estuário de Icapuí estavam parasitadas dezoito das cento e vinte ostras, correspondendo a uma prevalência média de 15,0%. As intensidades de infecção por *Perkinsus* sp. variaram de muito leve a leve nas duas localidades.

Palavras-chave: Manguezais; Ostra; Perkinsose; RFTM.

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Introduction

The coast of the semi-arid region of Brazil is known for the presence of large estuaries inhabited by numerous species of edible bivalve mollusks due to high primary productivity in seawater and favorable climatic and geographic conditions in these environments⁽¹⁾. Brazil is the second largest country in extension of mangrove areas, only lagging behind Indonesia⁽²⁾.

Two mangrove oyster species (*Crassostrea rhizophorae* and *C. gasar*) inhabit the estuaries of the Northeast region of Brazil⁽³⁾. The oyster *C. rhizophorae* is naturally found in estuarine environments along the Brazilian coast, fixed on substrates, mainly in the roots of mangroves⁽⁴⁾. Mangrove oysters are produced using artisanal techniques, which include the sampling of naturally settled spats at different places in an estuary⁽³⁾.

The production of oysters is threatened by the emergence of epizootic diseases, mainly caused by bacteria and protozoa⁽¹⁾. The bivalve mollusks may be affected by many parasites that can cause diseases and contribute to reducing natural, but mainly cultivated, populations⁽⁵⁾.

Studies on diseases in mollusks are important because some pathogens can affect and can cause massive mortality⁽¹⁾. Protozoa of the genus *Perkinsus* are important parasites of marine bivalves, sometimes causing significant pathological damage throughout the world^(6,7). Mortality events associated with this disease affect the dynamics of oysters populations and the structure and ecological function of beds of oysters, strongly modulated by environmental conditions⁽⁸⁾.

The infection caused by these protozoa is known as perkinsiosis or also as "Dermo", in reference to the first denomination of this protozoan regarding the oyster *C. virginica* in the USA⁽⁹⁾. Since the emergence of this genus, seven species of *Perkinsus* were confirmed and described as pathogens of marine snails all over the world⁽¹⁰⁾: *Perkinsus marinus*, *P. olseni*, *P. guqwadi*, *P. chesapeakei*, *P. mediterraneus*, *P. honshuensis*, and *P. beihaiensis*⁽¹¹⁾. Among these species, *P. marinus* and *P. olseni* require mandatory notifications to the World Organization for Animal Health, due to the risk that they represent for mollusk populations⁽¹²⁾.

In Brazil, four different species of *Perkinsus* have already been reported in mollusks: *P. chesapeakei*, *P. Beihaiensis*, *P. Marinus*, and *P. olseni*. They infect three species of hosts: *Crassostrea rhizophorae*, *C. gasar*, and *Anomalocardia brasiliiana*⁽¹³⁻¹⁵⁾.

The present article presents a study of the parasites *Perkinsus* sp. found in the mangrove oysters *Crassostrea rhizophorae* from Porto do Mangue estuary (Rio Grande do Norte state) and Icapuí estuary (Ceara state), two regions of the Brazilian semi-arid.

Material and Methods

Located in the semi-arid of Brazil, the coastal region of Ceara and Rio Grande do Norte presents hot humid tropical climate, with rains in the summer (December to March) and dry conditions during autumn, winter, and spring (April to November), and an annual mean air temperature of 32 °C. The city of Porto do Mangue, where the collections were held, comprises an area of approximately 332 km²(11). The city of Icapuí is located in the extreme east coast of Ceara state, in the sub-basin of the region of Baixo Jaguaribe, with an area of 423 km²(16). The two estuaries are covered by mangrove vegetation.

The first (*Crassostrea rhizophorae*) oyster samplings took place in Porto do Mangue estuary, in December (n = 10) of 2016, January (n = 40) and February (n = 40) of 2017, being carried out a preliminary assessment of the presence of *Perkinsus* sp. in Rio Grande do Norte state. Subsequently, oysters from Icapuí estuary in Ceara state were collected in September (n = 40), October (n = 40), and November (n = 40) of 2017 (Figure 1).

Oysters were collected manually and directly from the roots of the *Rhizophora mangle* (red mangrove) at low tide, and were transported alive in isothermal boxes to the laboratory facilities. Water temperature and salinity of the sampling area were measured.

Shells were measured (\pm standard deviation) in millimeters (mm). After that, they were kept in 20-liter aquariums in closed system with sea water and aeration for over 24h before processing for *Perkinsus* parasites isolation.

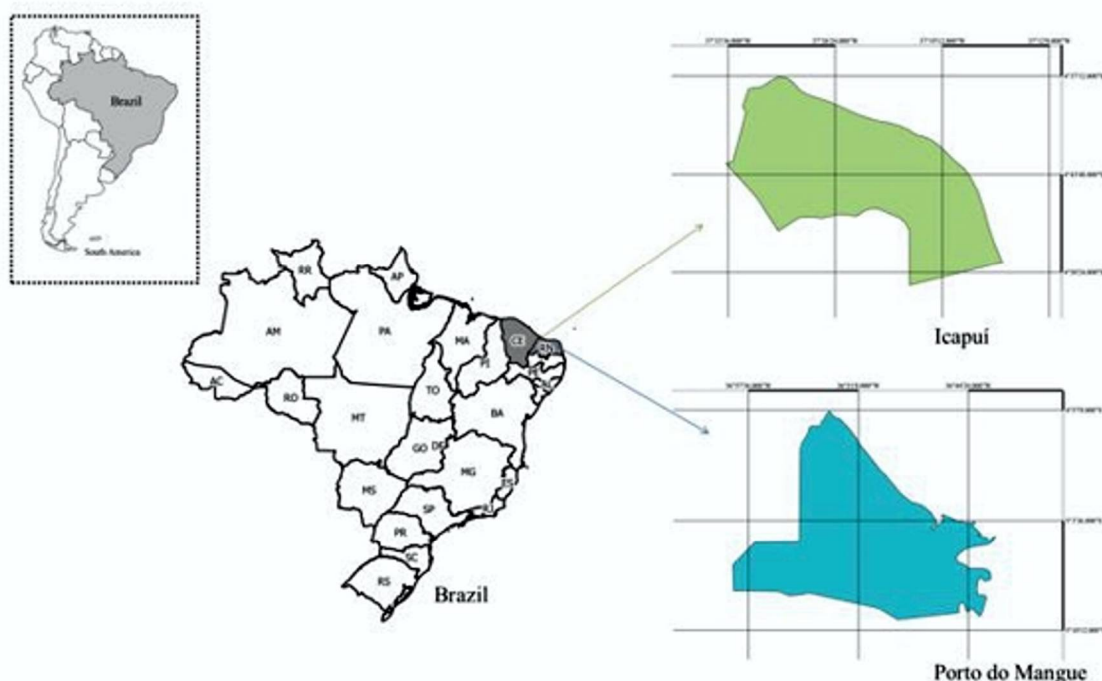


Figure 1: Brazil Map highlighting Ceara state and Rio Grande do Norte state, indicating the collection points at Porto do Mangue estuary and Icapuí estuary.

Pathological changes in the shell and body (mantle, gills, gonads, and digestive gland) were also observed. All animals collected in the estuary were processed in Ray's Fluid Thioglycollate Medium

– RFTM⁽¹⁷⁾.

Fragments of the gills were incubated in RFTM during seven days in the absence of light at room temperature. After the incubation period, the samples were prepared for microscopic analysis by cutting the fragments and macerating the tissue on a slide and examining the obtained preparations by optical microscopy to verify the presence of parasites of the genus *Perkinsus*.

The ecological and parasitological parameters, as the prevalence of pathogen (percentage of infected hosts, P%) and intensity of infection, were calculated according to Bush et al.⁽¹⁸⁾ and Silva et al.⁽¹¹⁾.

The scale of intensity of infection by *Perkinsus* sp. in the tissues of mangrove oysters (*C. rhizophorae*) was obtained in accordance with Sabry et al.⁽¹⁹⁾: *without infection* (without cells observed in the whole preparation); *very mild infection* (up to 10 cells observed in the whole preparation); *mild infection* (11 to 100 cells observed in the whole preparation); *moderate infection* (at least 40 cells observed in each of the 10 fields of the microscope (40x) scattered over the tissue); and *heavy infection* (more than 40 cells observed in each of the 10 fields of the microscope (40x) scattered over the tissue).

Results

Mean size (\pm SD) of oysters collected in Porto do Mangue estuary was 46.8 \pm 4.4 mm and in Icapuí estuary, 47.0 \pm 3.9 mm. Water temperature and salinity observed at the time of collection were 28 °C and 37‰ in Porto do Mangue estuary, respectively, and 27 °C and 35‰ in Icapuí estuary, respectively.

Five (n5) of the ninety (n90) oysters collected in Porto do Mangue estuary in December/2016, January and February 2017 were parasitized, which corresponded to a mean prevalence of perkinsiosis of 5.6%. The intensity of infection by *Perkinsus* ranged from very mild (n = 3) to mild (n = 2) in the months in study. December was the only month where values of prevalence and intensity of infection were not recorded because this month there were fewer individuals collected (Table 1). In Icapuí estuary, 18 (n18) of the 120 (n120) oysters collected were parasitized between the months of September, October, and November 2017, corresponding to a mean prevalence of 15.0%, and intensity of infection by *Perkinsus* ranged from very mild (n = 13) to mild (n = 5).

Table 1. Monthly indexes of prevalence (P%), intensity of infection by *Perkinsus* sp., and mean size (\pm standard deviation) of oysters (N – number of oysters collected; PM – Porto do Mangue; ICP – Icapuí)

Months of collection	N	Size (mm)	P%	Intensity of infection	Locality
December	10	44.2 \pm 3.5	0.00	without	PM
January	40	47.4 \pm 5.9	7.50	very mild and mild	PM
February	40	47.5 \pm 3.0	5.00	very mild	PM
September	40	48.1 \pm 4.3	10.0	very mild	ICP
October	40	46.3 \pm 3.7	22.5	very mild and mild	ICP
November	40	46.6 \pm 3.4	12.5	very mild and mild	ICP

The protozoan *Perkinsus* sp., belonging to the class Perkinsea, family Perkinsidae and genus

Perkinsus⁽²⁰⁾ after branchial process in RFTM, were identified by observation in optical microscope. The cells of the parasite were spherical and stained in black, and they presented a diameter from 28.23 μm to 51.30 μm (in both areas), typical characteristic of this species (Figure 2).

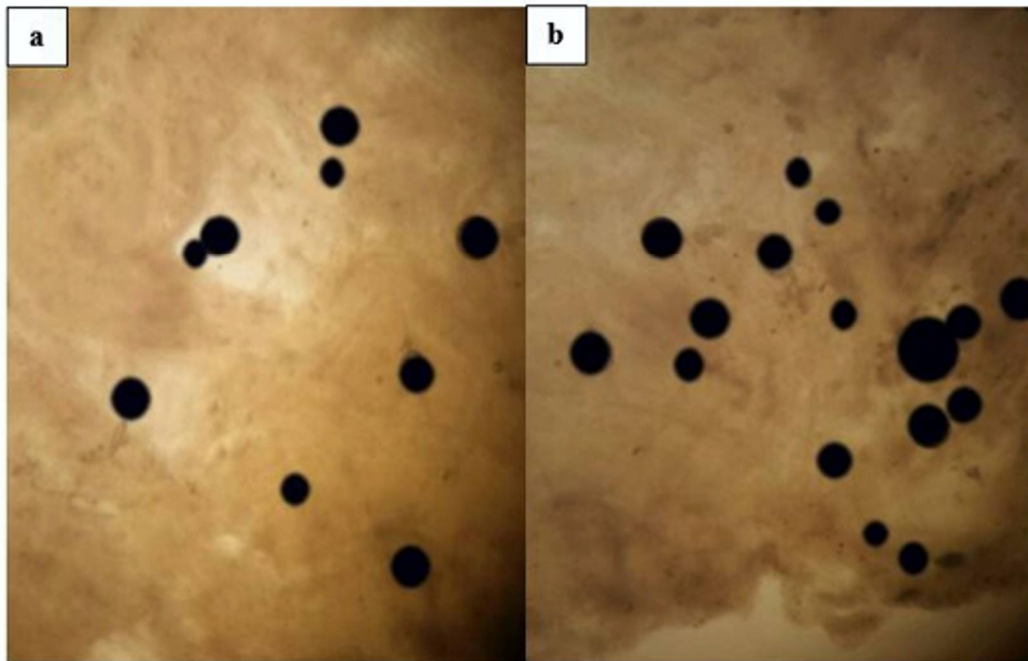


Figure 2. Hypnospores from *Perkinsus* sp. infecting the gills of *Crassostrea rhizophorae* after incubation in Ray's fluid thioglycollate medium (RFTM). *Perkinsus* sp. in *C. rhizophorae* (a) Porto do Mangue estuary, Rio Grande do Norte and (b) Icapuí estuary, Ceara.

Discussion

This is the first report of the occurrence of *Perkinsus* sp. parasitizing mangrove oysters (*Crassostrea rhizophorae*) in Porto do Mangue estuary (Rio Grande do Norte) and Icapuí estuary (Ceará). The preparation in RFTM revealed the presence of hypnospores of *Perkinsus* sp. infecting the gills of *C. rhizophorae* with very mild and mild infection intensity levels.

According to Boehs et al.⁽⁹⁾, protozoa of the genus *Perkinsus* affecting bivalve mollusks in various regions of the world and can cause major economic losses depending on the species and the affected host, requiring further studies to monitor this epizootiology.

The prevalence of infection by *Perkinsus* was considerably lower than that found in studies with mollusks from other regions of Brazil. Sabry et al.^(19,21) found a prevalence of 5.78 and 23.33% in *C. rhizophorae* in the estuary of Pacoti on the coast of Ceara state. On the coast of Bahia state, the high prevalence of *Perkinsus* sp. in *C. rhizophorae* has reached 63%⁽²²⁾. Queiroga et al.^(15,23) recorded high (93.3%) and moderate (48.9%) prevalence in *C. gasar* in the estuary of the Mamanguape River (Paraíba state). Silva et al.⁽¹³⁾ verified 100% (high) prevalence of *P. marinus* infecting *C. rhizophorae* in Paraíba River (Paraíba state). Ferreira et al.⁽¹⁴⁾, in the estuary of Timonha river (Ceara state), obtained a mean prevalence of 14.7% of *Perkinsus* sp. infesting *Anomalocardia brasiliiana*. Scardua et al.⁽³⁾, in São Francisco river, Sergipe state, obtained a moderate prevalence of 47.8% in *Crassostrea* spp.. Silva et al.⁽¹⁾ studied the presence of *Perkinsus* on a farm in Rio Grande do Norte state and

obtained 17 to 75% prevalence. Silva et al.⁽²⁴⁾ was also detected in *P. marinus* in *C. gasar* from Sergipe state; the mean prevalence was higher in cultured oysters (56%) than in wild oysters (35%).

The prevalence in this study was higher than that found by Neto et al.⁽¹²⁾, who observed the prevalence of *P. chesapeaki* (2.6%) and *P. beihaiensis* (3.3%) infesting *C. rhizophorae* in the estuary of Jaguaribe river (Ceara state).

Perkinsus parasite was originally described as a pathogen of abalone in South Australia and the first record of *Perkinsus* in South America was made in Uruguay⁽²⁵⁾.

According to Silva et al.⁽²⁴⁾, the dynamics of infection by *P. marinus* and *P. olseni* in *C. gasar* (= *C. brasiliiana*) may have seasonal variations in tropical climate and be associated with cultural practices. This variation of infection may also be associated with *C. rhizophorae*, which would explain the low prevalence in the study.

In the semi-arid region of Brazil, the pattern of infection by *Perkinsus* sp. in oysters seems to be associated with salinity, i.e. the prevalence decreases at low salinities, which usually occurs in the rainy season^(1,15,22,24). Accordingly, the prevalence on this study was higher in the Icapuí estuary in the months characterized by drought, and low in Porto do Mangue estuary during rainy months.

Molecular biology analysis to confirm phylogenetic and provide a molecular diagnosis to identify species of *Perkinsus* in mollusks from Rio Grande do Norte and Ceara are still necessary.

According to Sabry et al.⁽²¹⁾, the effects of perkinsosis on the host and the natural populations of oysters from the northeast region should be evaluated. It is imperative to introduce strategies to restrict the spread of the disease.

Ferreira et al.⁽¹⁴⁾ states that, due to the large differences observed in the prevalence of *Perkinsus* sp. in mollusks of the entire Brazilian coast, further studies are needed to better understand the dynamics of this parasitic pathogen infection in mangrove oysters.

In conclusion, this is the first study that identifies *Perkinsus* sp. naturally infecting the mangrove oysters (*Crassostrea rhizophorae*) in Porto do Mangue estuary (Rio Grande do Norte state) and Icapuí estuary (Ceara state) located in the semi-arid region of Brazil with intensity levels of infection varying from very mild to mild.

Ethical Approval

All procedures performed in this study involving animals were in accordance with the ethical standards of the institution.

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