Influence of the copepod *Mesocyclops longisetus* (Crustacea: Cyclopidae) on the survival of *Vibrio cholerae* O1 in fresh water

Influência do copépode *Mesocyclops longisetus* (Crustacea: Cyclopidae) na sobrevivência de *Vibrio cholerae* O1 em água doce

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Abstract *In an experimental microcosm, an analysis was performed of the influence exerted by freshwater* Mesocyclops longisetus *copepods on the survival of* Vibrio cholerae *O1 serovar Inaba. In the State of Ceará, copepods are used in the control of* Aedes aegypti *larvae. The system consisted of water with a salinity of 0.27‰ and pH 7.5, which after sterilizing filtration was distributed into seven flasks with a volume of 400 ml; in each of six flasks, 10 live copepods were inoculated along with 1 ml of an 8-hour culture of* Vibrio cholerae *O1 at 37ºC in Alkaline Peptone Water, resulting in a concentration of 3.80x10⁴ colony-forming units. The control flask contained only the water with the same bacterial suspension. The system was maintained for six days at room temperature (25-28ºC), and daily duplicate counts were performed in TCBS Agar. Results confirmed a clear association between* Vibrio cholerae *O1 and the live copepods, based on survival of the bacteria at compatible levels with the initial inoculation until the sixth day of the analysis.*

Key words Vibrio cholerae*; Copepods; Cholera; Water Microbiology*

Resumo *Foi analisada num microcosmo experimental a influência exercida por copépodes de água doce da espécie* Mesocyclops longisetus *na sobrevivência de* V. cholerae *O1 sorovar Inaba. Os copépodes são utilizados no controle de larvas de* Aedes aegypti *no Estado do Ceará. O sistema estava constituído de água com salinidade de 0,27‰ e pH 7,5, que após filtração esterilizante foi distribuída em sete frascos em volumes de 400 ml, sendo que em cada um dos seis frascos foram inoculados dez copépodes vivos e 1 ml de uma cultura de oito horas a 37ºC em água peptonada alcalina (pH 8,5) de* V. cholerae *O1, resultando uma concentração de 3,80x10⁴ unidades formadoras de colônias. O controle continha apenas a água com a mesma suspensão bacteriana. O sistema foi mantido durante seis dias à temperatura ambiente (25 – 28ºC) e, diariamente, foram realizadas as contagens em duplicata em Ágar TCBS. Os resultados evidenciaram uma nítida associação de* V. cholerae *com os copépodes vivos, através da sobrevivência bacteriana em níveis compatíveis com o inóculo inicial até o sexto dia da análise.*

Palavras-chaves Vibrio cholerae*; Copépodes; Cólera; Microbiologia da Água*

Introduction

The ecology of *Vibrio cholerae* in an aqueous environment is highly influenced by the ability of cells to adhere to specific surfaces in order to create a better microenvironment. Chitin, the main component of crustacean carapaces, has been found to be an appropriate surface for the adherence and survival of *Vibrio cholerae* (Dastidar & Nayaranaswami, 1968). The role of zooplankton, specifically copepods, in the survival and multiplication of *Vibrio* in an aqueous environment is of great importance to its natural history (Colwell & Spira, 1992).

Huq et al. (1983, 1984) showed that the survival of *Vibrio cholerae* O1 is enhanced when it is cultured with laboratory-grown planktonic copepods originally isolated from fresh and estuarine waters. The association and growth of *Vibrio cholerae* on copepods reach an optimum condition in microcosms with 1.5% salinity, pH 8.5 and a temperature of 30ºC.

Tamplin et al. (1990) showed that clinical strains of *Vibrio cholerae* O1 were attached preferably to zooplankton molts (exuviae) rather than to whole specimens.

Copepods have been used for the biological control of mosquitoes, specially *Aedes aegypti* L., the vector for both dengue and urban yellow fever. Therefore, copepods of the *Mesocyclops longisetus* Thiebaud specie were used to control first instar larvae in the village of Preaoca in the State of Ceará, Brazil. The copepods were placed in clay pots and concrete tanks built in bathrooms and wells (Cabral et al., 1993). Copepods have been used to control mosquito larvae in the French Polynesian islands (Rivière et al., 1987; Lardeux et al., 1992); in Queensland, Australia (Brown et al., in press); in New Orleans, US (Marten, 1990); and in Honduras (Marten et al., 1994a, 1994b).

With the recent cholera epidemic in the State of Ceará (1993), analyzing the influence of such biocontrol agents is strongly recommended – since it is intended for domestic use to control mosquito larvae – on the survival of *V.cholerae* O1 in fresh water and thus the transmission of the agent by ingestion of water without prior treatment.

Material and methods

Sample collection

Experiments have been carried out using fresh water collected from an artificial reservoir (Santo Anastácio dam) located in the Pici campus of the Federal University in Ceará. The water was then filtered in cotton to expunge the surplus of organic matter composed mainly of blue-green algae and subsequently sterilized by filtration through a 0.22 mm Millipore® membrane. Parameters for the water were as follows: pH 7.5, salinity 0.27‰ (Strickland & Parsons, 1972), and temperature 25ºC.

The copepod specimens were collected from bodies of water near the city of Fortaleza, Ceará, and identified as *Mesocyclops longisetus* (Reid, 1985). After identification, a pure culture of the species was developed from a single gravid female (Suarez et al., 1992).

Copepods were washed in one liter of a sodium hypochloride solution (Na OCl), at a concentration of 30 ppm, for a period of 15 minutes to avoid the removal of surface bacteria.

Bacterial strain and culture methods

The strain of bacteria used was clinically isolated from the Laboratório Central de Saúde Pública of the State of Ceará and identified as *V.cholerae* O1 serovar Inaba and biotype El Tor. The bacterial strain was grown in Alkaline Peptone Water (Bacto-peptone-Difco 1% (w/v) and NaCl 1% (w/v) at pH 8.5) for a period of 8 hours at 37ºC (Ministério da Saúde, Comissão Nacional de Prevenção da Cólera, Subcomissão Nacional de Diagnóstico Laboratorial, 1992).

Procedures for survival studies

In a series of seven erlenmayer flasks containing 400 ml of filter-sterilized water, each of six flasks was inoculated with ten live copepods and 1 ml of a *V.cholerae* O1 culture to a final concentration of approximately 3.80×10^4 colony-forming-units (CFU/ml). The seventh flask was used as control with the same concentration of water and bacteria. The flasks were incubated at room temperature (25-28º) under static conditions.

To assess the data, 1ml of water was taken from each of the flasks and appropriate decimal dilutions in saline solution (0.85%), 0.1ml aliquots were spread onto duplicate TCBS Agar plates (Thiosulphate-citrate-bile salts-sucrose, Difco) and were incubated at 37ºC for 24 hours. Sucrose positive colonies (yellow) with the typical characteristics of *Vibrio* were enumerated and picked for biochemical and serological identification by following accepted criteria (Hofer, 1974).

For sampling, the life copepods were withdrawn from water, dried out, crushed, and ho-

mogenized manually with 1 ml of saline solution. After appropriate decimal dilutions in saline (0.85%), the same methodology described was used for vibrio enumeration and characterization.

Results and discussion

In the model we adopted, an attempted analysis was performed to determine the survival capacity of *V. cholerae* O1 in the presence of the *Mesocyclops longisetus* copepod in a fresh aqueous microcosm.

The data collected from the counting of colony-forming units during the total span of the experiment proved that the microcrustaceans probably served as an adherence substrate for *V. cholerae*. This assertion is supported by the results observed in the counts performed in the live copepods, in the water containing them, and in the control (Table 1 and Figure 1).

As an attempt to better express the observed phenomenon, a link was established between the bacterial population counts verified in the copepods and in the control. In this case, it should be pointed out that the maximum adherence achieved by *V. cholerae* on the crustacean occurred on the fourth day (Table 1 and Figure 1), representing an increase of approximately 77 times in the concentration of adhered vibrios. lnteresting enough, one day later this rate decreased significantly, but it stíll remained 27 times higher than the control. Most probably this occurrence was due to the death of some copepods that had not been collected for counting. It could be that an animal carapace, whích consists basically of chitin, influenced the environment with more nutrients, therefore enhancing the viability of the vibrio, given its capacity to use this polysaccharide as a source of carbon (Nalin et al., 1979). This hypothesis is confirmed by the absence of bacteria in the water of the control flask on the 6th day, which was still observed even after it was partially replaced by an adequate culture medium, in this case was Alkaline Peptone Water – pH 8.5. This maneuver also eliminates the possibility of the microorganism being adhered to the inner surface of the flask and demonstrates that the physical-chemical conditions of the water, which are considered ideal by several authors, had relatíve influence in the survival of *V. cholerae* O1. It is evident that these abiotic parameters in an experimental microcosm are decisive for maintaining bacteria viability in the initial phase, that is, in the first 24 hours of the investigation, in which the most important factor is the pH in the alkaline range. All the water samples tested with pH in the acid range, generally around $\leq$ 6.5, notwithstanding the compatible salinity content, did not allow for the survival of the choleric vibrio after 24-hour observation.

Undoubtedly the ecology of this human enteropathogenic microorganism, indigenous to the aqueous environment, has remained obscure ever since the initial speculations made by Koch in 1884, although some constituents of the ecossystem have been recognized as reservoirs for the bacteria in an attempt to explain cholera's seasonal occurrence in endemic areas (Islam et al., 1993).

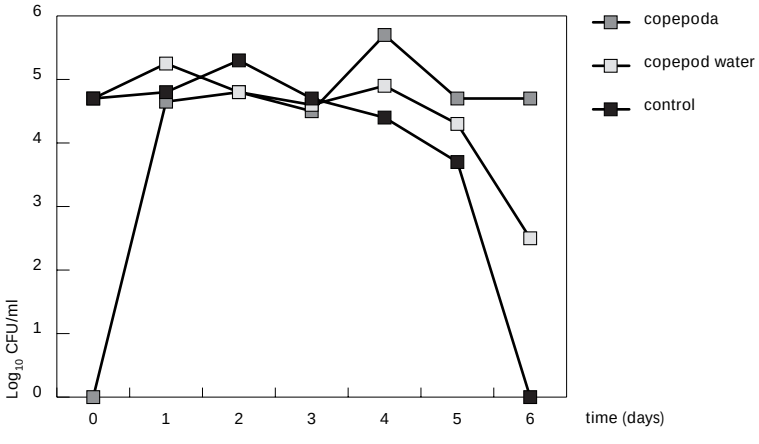
Table 1

V.cholerae O1 count expressed in colony-forming units (CFU/ml), in live copepods, in water and control.

Time (days)	CFU/ml		
	Control	Copepod water	Copepods
0	3.80×10^4	3.80×10^4	–
1	5.70×10^4	1.60×10^5	4.80×10^4
2	2.0×10^5	7.10×10^4	7.30×10^4
3	3.25×10^4	2.79×10^4	1.91×10^4
4	7.84×10^3	4.70×10^4	6.01×10^5
5	1.32×10^3	1.03×10^4	3.48×10^4
6	–	20	3.27×10^4

Figure 1

Survival of *Vibrio cholerae* O1 associated with copepods in the laboratory microcosms.



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