RESEARCH NOTE

Fate of *Bacillus sphaericus* after Ingestion by the Predator *Belostoma micantulum* (Hemiptera: Belostomatidae)

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The persistence of *Bacillus sphaericus* in the environment can be influenced by the activity of other organisms present in the mosquito larval habitat and its controling efficacy can be related to the density of non-target arthropods (S Karch et al. 1990 *J Am Mosq Control Assoc* 6: 47-54).

Belostoma micantulum, commonly known as water-bug, is an aquatic hemiptera prevalent in South America from Guiana to Argentina (GR Spinelli et al. 1983 *Neotropica* 29: 27-34) which coexists with mosquito larvae and is an efficient predator of them (AS Mijares, RG Broche 1985 *Rev Cub Hyg y Epid* 37: 203-209).

With the aim of assessing the fate of the bacteria after their ingestion by the hemiptera as well as their behavior in cadavers of these insects, 90 adults of *B. micantulum*, each placed in a plastic cup containing 200 ml of dechlorinated water was fed each with ten 4th instar larvae of *Culex quinquefasciatus* previously infected with *B. sphaericus* 2362 strain (8.1×10^4 spores/ml for 30 min). A group of 50 mosquitoes larvae was transferred to a cup containing only distilled water to confirm the bacterial infection showed by observation of 100% of mortality after 48 hr. Forty-five hemiptera were killed by crushing their heads immediately after feeding on larvae and were kept isolated in plastic cups in sterile dechlorinated water. The rest of the hemiptera was kept alive in the same conditions. Starting from the first day after feeding the water-bugs, their guts were dissected, ground and homogenized in 1 ml of sterile dechlorinated water. This material was split into two subsamples, one of which was submitted to a heat shock (80°C/15 min) to kill all vegetative cells and nonspore-forming bacteria. Of each subsample, 0.1 ml or a dilution of it was plated in NYSM medium (PS Myers, AA Yousten 1978 *Infect Immun 19*: 1047-1053) in Petri dishes containing 100 mg/l of streptomycin. Three hemiptera were dissecated in each day and their guts were individually ground, homogeinized in water and plated in culture medium. The colony counts were recorded after a 24 hr growth period at 35°C. *B. sphaericus* presence was confirmed by the observations of morfology colonies as well as microscopic observation of smears stained by the Gram's method. Also, the material of some randomly selected colonies of each dish was diluted in water and transferred to plastic cups each containing dechlorinated water and ten 4th instar larvae of *Cx. quinquefasciatus* to confirm entomopathogenicity after 48 hr. All larvae died after this period of time.

Figure shows the observed results. The number of colony-forming units (CFU) and spores found in the gut of live water-bugs decreased to very low levels in the first days after ingestion of infected mosquito larvae. After 30 and 60 days no more bacterial presence was detected (Fig. A). The platings of feces of water-bugs collected in the cups which contained them, in the first days of experiment, were also positive for *B. sphaericus*, showing that this bacteria is released through water-bugs feces during the first days after ingestion of infected mosquitoes larvae. In water-bugs cadavers, after a little initial decrease, we observed a strong increase of CFU number and spores, that showed to be stable from the 10th until the 60th day when the experiment was finished (Fig. B). It seems that these bacteria are able to make use of water-bug cadavers in order to grow and posteriorly esporulate.

B. sphaericus grows in dead larval tissues of *Cx. quinquefasciatus* larvae (S Silapanuntakul et al. 1983 *J Invertebr Pathol* 42: 387-392, B Des Rochers, R Garcia 1984 *Mosq News* 44 (2-Part I): 160-165). The present work confirms the hypothesis that, in the absence of dead mosquito

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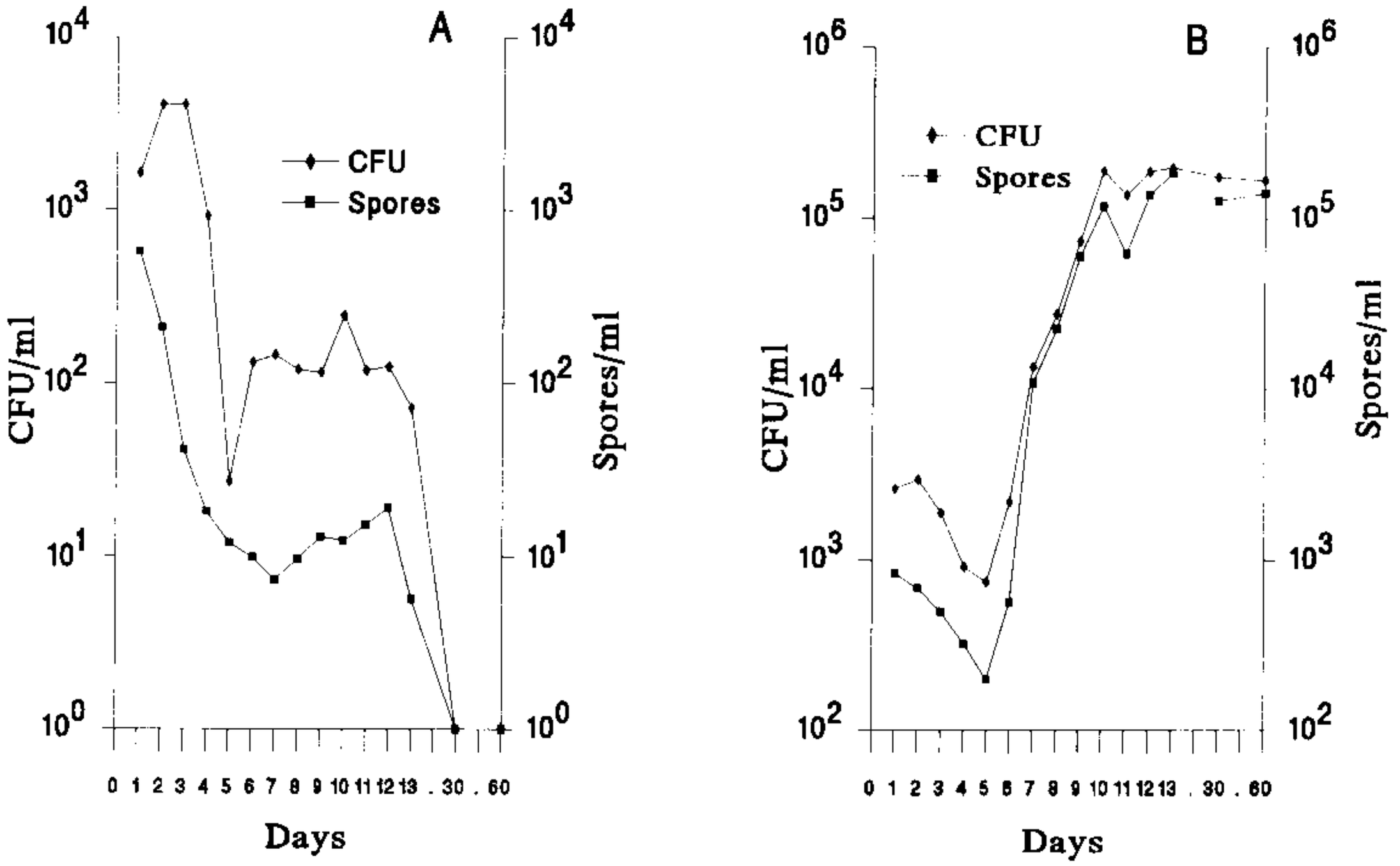

Number of colony-forming units - CFU (◆) and spores (■) of *Bacillus sphaericus* in the guts of alive (A) and dead (B) *Belostoma micantulum* fed with *Culex quinquefasciatus* larvae infected by *B. sphaericus* for different periods of time. Each point represents the mean of three observations.

larvae, this bacteria can still multiply if sufficient proteinaceous material is available in its environment (VL Kramer 1990 *J Econ Entomol* 83: 1280-1285). This replication in cadavers may occur in other animals which, somehow, ingest *B. sphaericus*.

The ability of *B. sphaericus* to reproduce in cadaveres of other animals besides mosquito larvae could be an important factor to increase its persistence in mosquito breeding sites and consequently result in its better efficacy as a larval control agent.