THE EFFECT OF *BACILLUS SPHAERICUS* UPON THE SUSCEPTIBILITY OF *ANOPHELES QUADRIMACULATUS* TO *PLASMODIUM BERGHEI*[1]

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ABSTRACT. Larvae of *Anopheles quadrimaculatus* were exposed to *Bacillus sphaericus*. The surviving adults took blood meals on hamsters infected with *Plasmodium berghei*. Fewer mosquitoes were infected than were the paired controls. The inhibitory action appeared to occur during the early stages of the infection in the mosquito gut.

Bacillus sphaericus is a facultatively parasitic, aerobic, spore-forming bacterium, several strains of which are lethal to larval mosquitoes. The lethal factor is a toxin which damages or destroys the gut epithelium. Higher dosages of *B. sphaericus* are required for the control of *Anopheles* spp. than *Culex* spp. (Lacey and Singer 1982, Lacey et al. 1986). Although there is no active infection in adults which survive sublethal dosages as larvae, there might be residual effects upon mosquitoes which could result in a reduction in their capacity as malaria vectors. As evidence of this, *B. sphaericus* has been shown to reduce the susceptibility of an Asian malaria vector, *Anopheles stephensi* Liston, to *Plasmodium yoelii* (Noireau and Karch 1983).

Anopheles quadrimaculatus Say, the principal vector of human malaria in the southeastern United States, is also susceptible to *P. berghei*, the causative agent of a rodent malaria, and thereby may be used as an experimental malaria system.

The object of these experiments was to determine if *An. quadrimaculatus* adults which survived exposures to *B. sphaericus* as larvae have reduced susceptibility to *P. berghei*.

Between 2,000 and 5,000 newly hatched *An. quadrimaculatus* larvae from the colony at the Gainesville, FL, United States Department of Agriculture laboratory were placed in 43 × 60 cm trays containing 3 liters of well water and 1 gm of equal parts liver powder and brewer's yeast. Two days later another gram of the liver powder and yeast was provided; and on the 4th and 5th days they were fed 2 gm of equal parts liver powder, brewer's yeast and hog chow supplement. Additional feedings were provided as necessary while the larvae were pupating on days 6–8.

The 4-day-old, third instar larvae were treated with *B. sphaericus* (2362 IF 121), at the rate of 3 mg per tray (1 ppm). Two days later the larvae were rinsed to remove the dead larvae and remaining *B. sphaericus* and returned to clean trays. During the rinsing process, the total volume of the *B. sphaericus* treated and control larvae in each tray was measured. To do this, the contents of each tray were poured into a large funnel and gently swirled. The dead larvae sank and were drawn off the bottom, after which the live larvae were decanted through a graduated cylinder with a screen on the bottom. The volume of the treated larvae, divided by the volume of the untreated larvae (each the mean of 2–4 trays), was used as an approximate mortality rate. Only the treatments producing 40–60% mortality were kept for experimental use.

Pupae were picked, either by the ice water method (Hazard 1967) or by suction, transferred to plastic cups with 200 ml clean well water, and placed in 46 × 38 × 37-cm cages. The cages of adult mosquitoes were kept in an insectary maintained at 20 ± 1°C and high RH. Carbohydrates were supplied to the adults in the form of a 10% sucrose solution. Two to five days after emergence, female mosquitoes were removed from the large cages by attraction to emissions from a technician's hand to assure that the experimental insects were physiologically ready to take a blood meal. Between 100 and 200 mosquitoes were transferred to 22-cm^3 cages in which they were kept for the subsequent procedures. Control mosquitoes were treated identically except that they were not exposed to *B. sphaericus*.

Plasmodium berghei NK 65 was induced in hamsters by injection of infected blood.[4] When the parasitemia was in the 3rd to 5th day, usually the 4th, the rodents were lightly anesthetized and laid on the screened top of the cage with mosquitoes. One cage each of treated and control

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[4] The guidelines for the care and use of laboratory animals of the N.I.H., U.S. Public Health Service were followed.

mosquitoes were fed on each *P. berghei*-infected hamster for a total of 98 paired trials. The unfed mosquitoes were removed from the cage. Ten percent sucrose was supplied for additional nutrition. A blood meal on an uninfected animal was offered 1 week later.

After 12–14 days of incubation, mosquitoes were dissected and examined for evidence of infection as shown by the presence of oocysts on the gut. A total of 1,906 guts were examined, and both the number of infected guts and the estimated number of the oocysts on the infected guts were recorded. After eliminating the 20 paired lots in which neither the control nor experimental lots contained infected individuals, there were 1,508 specimens evaluated.

The infections in the control and test mosquitoes were compared (Table 1). The mosquitoes treated with *B. sphaericus* were significantly less susceptible to infection than the controls ($P = 0.03$ using the Fisher's Exact Test, 1-tail). In terms of oocyst counts, both groups were about the same ($P = 0.98$) (Table 2).

The effect of *B. sphaericus* on the malaria infection apparently was on the early stages of the infection in the mosquito. After the infections were well-established as oocysts by day 10, there appeared to be no effect on the oocyst count as the densities were about the same in the treated and the control groups (Table 2).

In a limited experiment involving a total of 102 insects of which 38 were in the test group, using a different malaria (*P. yoelii*) and a different malaria vector (*An. stephensi*), Noireau and Karch (1983) obtained results similar to ours in terms of infection rates but also found the density of the oocysts to be significantly ($P < 0.05$) lower in the *B. sphaericus*-treated mosquitoes than in the controls.

Table 1. Susceptibility to *Plasmodium berghei* of *Anopheles quadrimaculatus* exposed to *Bacillus sphaericus* compared to the untreated controls in 78 paired lots.

	Mosquito guts examined for oocysts		
	Negative	Infected	Total
Controls	373	381	754
Exposed	410	344	754
Totals	783	725	1,508

Fisher's Exact Test (1-Tail) $P = 0.03$.

Table 2. Oocysts densities in the *Bacillus sphaericus* treated and control mosquitoes.

	Oocysts per infected gut		
	1–25	Over 25	Totals
Controls	291	90	381
Treated	263	81	344
Totals	554	171	725

$P = 0.98$.

The relatively high dosages of *B. sphaericus* required (Lacey and Singer 1982) and the incomplete larval mortality in field trials (Lacey et al. 1986) might not represent the entire effect on malaria suppression. Our results show a significant reduction of susceptibility to malaria of the adults surviving from the larvae exposed to *B. sphaericus*. This should be included in the assessment of the total effect of a bacterial toxin in a vector control program.

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