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EFFECT OF *BIOMPHALARIA ALEXANDRINA* SNAILS INFECTED BY *BACILLUS THURINGIENSIS KURSTAKI* ON THREE SUCCESSIVE GENERATIONS OF *SCHISTOSOMA MANSONI*

[37]

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ABSTRACT

The effect of infection of *Biomphalaria alexandrina* snails with *Bacillus thuringiensis kurstaki* on various stages of *Schistosoma mansoni* life cycle was studied for three successive generations. Thus, two groups of snails were exposed to a sublethal concentration of the bacteria (0.08 gm/L water) containing 32000 IU/mg, for one week and to schistosome miracidia. One group was exposed to the miracidia before bacterial infection, while the other group to the miracidia after the bacterial infection. Cercariae produced from each group of snails were used to infect albino mice. The infection of snails and mice with the parasite was repeated for three generations of the parasite. In the first case, data obtained show that the schistosome infection rate of snails was considerably reduced being 60%, 18%, and 66.6% versus 90 % , 92% and 90% in untreated control snails in the three generations of the parasite, respectively. Meanwhile, the mean prepatent period was extended being 29.1 ± 4.3 days, 33 ± 1 days and 38.5 ± 2.5 days versus 27 days in the control group. The number of worms recovered from infected mice showed reduction of 52 %, 78.4% and 58.6%, respectively. In the second case, the infection rate of snails was 40%, 16% and 73.7% for the three successive parasite generations and the prepatent period was 32 ± 1 days, 32 ± 2.3 days and 35 ± 2.8 days, respectively. The reduction percentage of the recovered worms was 34.8, 73.6 and 72.9 in the sccessive generations, respectively. The present results prove that infecting *B.alexandrina* snails with a sublethal concentration of *B. thuringiensis kurstaki* bacteria exhibits clear negative effect on the transmission of *S. mansoni* in three successive generations. So, it could be recommended to use *B. thuringiensis kurstaki* as a potential biocontrol agent against *S. mansoni*.

Key words: Biomphalaria alexandrina, *Bacillus thuringiensis* kurstaki, *Schistosoma mansoni*, Schistosome infection, Worm recovery

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INTRODUCTION

Old references show that *Biomphalaria alexandrina*, the snail host of *Schistosoma mansoni* in Egypt, had been widely distributed in the Nile Delta only (**Barlow** & **Munech**, **1951**), while recent publications show that this snail has invaded upper Egypt till Aswan (**Heynman, 1978**). It is known that snail control is the most promising method of supressing schistosomiasis transmission, and utilizing molluscicides has proved to be the single most effective agent of reducing snail population (**Shiff, 1961**). However, chemical means are hazardous from the environmental point of view. Using biological agents should have advantages over the chemical methods, being safer and more specific. One of these agents is bacteria which is specially pathogenic against the target snails (**Madsen, 1992**).

In this respect certain species and varieties of bacteria such as *Vibrio, Pseudomonas, Citrobacter, Aeromonas,* and *Bacillus thuringiensis* have been tested on some biological aspects of fresh water snails (**Ducklow *et al* 1980** and **1981**; **Larget & Barjac, 1981**; **Cheng, 1986**; **Madsen, 1992**; **Osman *et al* 1992**; **Singer *et al* 1994**; **El Emam *et al* 1996**; **El Emam & Haroun, 1999**). *B. thuringiensis kurstaki* was found to have a significant toxic effect on *B.alexandrina* snails and a concentration of this bacteria as low as 0.08 gm/L water has a considerable reducing effect on egg production of the snail and on its infection with *S.mansoni* (**Gamal *et al* 2000**).

The aim of the present work was to study the effect of infection of *B. alexandrina* snails with *B. thuringiensis kurstaki* on stages of the life cycle of three successive generations of *S. mansoni.* Exposure of snails to the bacteria was performed before and after infection of snails with the schistosome miracidia. This study should contribute to the evaluation of utilizing this variety of bacteria as a biocontrol agent against the present important snail vector of schistosomiasis.

MATERIAL AND METHODS

Laboratory produced *Biomphalaria alexandrina* snails of the same diameter (3-5 mm) and albino mice (CD1) were obtained from the Schistosome Biological Supply Program (SBSP), Theodor Bilharz Research Institute (TBRI), Egypt. The commercial bacteria *Bacillus thuringiensis kuristaki* (Ecotech bacteria) in the form of a powder containing 32000 IU/mg was obtained from the Plant Protection Institute, Ministry of Agriculture, Dokki.

One hundred snails were divided equally into two groups. Snails of one group were exposed to *S. mansoni* miracidia then after they were maintained for one week in two aquaria containing Ecotech bacteria water solution (0.08g/L) i.e. 2560000 IU/L., 25 snails in 2 L of bacterial solution in each aquarium. Such concentration has been found to be sublethal to *B. alexandrina* snails (**Gamal *et al* 2000**). After that snails were washed carefully and transferred to clean dechlorinated water. The other group of snails was maintained in the same concentration of bacteria water solution for one week, then thoroughly washed with running declorinated water, and exposed to *S. mansoni* miracidia.

Exposure of snails of both groups to miracidia was in mass using 8-10 miracidia / snail for two hours. Only freshly hatched miracidia were used within one

hour of hatching (**Prah and James, 1977**). Twenty-five snails were infected with *S. mansoni* miracidia without exposure to the bacteria to be used as control. Bacteria infected and control snails were maintained under standardized laboratory conditions (**Liang, 1974**). Starting from 26 days post infection, the surviving snails were examined individually twice weekly for cercarial shedding. The shedding snails were counted and separated in other aquaria. The produced cercarial suspension from both groups of snails were used to infect six albino mice. Each one was exposed by tail immersion technique to 80-100 cercariae in 5ml of declorinated tap water at room temperature (25±1°C.) for 2 hours.

Infection of snails and mice was repeated three times using the same procedures to produce three generations of the schistosome parasite. In each generation the snail infection rate and prepatent period of the parasite in snails were determined. Worms were recovered from the portomesenteric system of mice by the perfusion technique 45 days post infection and the mean recovery rate of worms was calculated in each case.

RESULTS

In the first case, in which the snails were exposed to Ecotech bacteria after being infected with *S. mansoni* miracidia, the results (Table, 1) indicate that the tested bacteria caused a considerable reduction in the infection rate of snails in the three parasite generations. The highest reduction (80.4%) was recorded in the second generation. Meanwhile, the prepatent period of the parasite in snails was prolonged in the successive generations, being 29.1 ± 4.3 days, 33 ± 1 days and 38.5 ± 2.5 days, respectively, showing increasing change from the first (7.8%), second (22.2%) to the third (42.6%) generations. In the second case in which exposing of snails to bacteria was performed before miracidial infection, the bacteria caused significant reduction (Table, 2) in the infection rate in the 1st and 2nd generations. While the reduction was much less in the 3rd generation. The length of the prepatent period of the parasite in snails increased almost equally in the three generations of the parasite.

Comparing results of both cases, it appears that the infection rate of *B. alexandrina* with *S. mansoni* was more reduced in the first generation of the second case, and almost similar in the next two generations. The prepatent period was slightly longer in the 1st case than the 2nd one.

The cercariae produced from *B. alexandrina* snails exposed to bacteria before and after infection with *S. mansoni* miracidia, produced significantly less numbers of worms from the mice in the successive three generations (Tables 3 & 4) in comparison with those produced from the control group, without significant different between male and female worms. The difference in recovery rate of worms between the three generations of parasite and in both cases of bacterial infection is slight and insignificant.

DISCUSSION

Several reports are found in the literature on the impact of various spp. and strains of *Bacillus* bacteria on schistosome vector snails, showing different

Table 1. Effect of infecting *Biomphalaria alexandrina* snails with *Bacillus thuringiensis kurstaki* after *Schistosoma mansoni* miracidial exposure on infection and intramolluscan development of the parasite in three successive generations

Parameter	First generation			Second generation			Third generation		
	Control	infected with bacteria	% of change	Control	infected with bacteria	% of change	Control	infected with bacteria	% of change
Number of exposed snails	25	25		50	50		30	30	
% of Infection	90	60	(-33.3)	92	18	(-80.4)	90	66.6	(-26)
Mean prepatent period (days)	27	29.1	(+7.8)	27	33	(+22.2)	27	38.5	(+42.6)
SD	0.3	4.3		1.4	1.0		0.7	2.5	

Table 2. Effect of infecting *Biomphalaria alexandrina* snails with *Bacillus thuringiensis kurstaki* before *Schistosoma mansoni* miracidial exposure on infection and intramolluscan development of the parasite in three successive generations

Parameter	First generation			Second generation			Third generation		
	Control	infected with bacteria	% of change	Control	infected with bacteria	% of change	Control	infected with bacteria	% of change
Number of exposed snails	25	25		50	50		30	30	
% of Infection	90	40	(-55.5)	92	16	(-82.6)	90	73.7	(-18.1)
Mean prepatent period (days)	27	32	(+18.5)	27	32	(+18.5)	27	35	(+29.6)
SD	0.3	1		1.4	2.3		0.7	2.8	

Table 3. Effect of infecting *Biomphalaria alexandrina* snails with *Bacillus thuringiensis kurstaki* after *Schistosoma mansoni* miracidial exposure on worm recovery of the parasite in three successive generations

Worm sex	Control	1st generation	2nd generation	3rd generation
	Mean number of recovered worms/mous	% reduction of recovered worms	% reduction of recovered worms	% reduction of recovered worms
Female	38.7	48.3	76.7	70.3
Male	44.6	55.2	79.8	48.4
Total	83.3	52	78.4	58.6

Table 4. Effect of infecting *Biomphalaria alexandrina* snails with *Bacillus thuringiensis kurstaki* before *Schistosoma mansoni* miracidial exposure on worm recovery of the parasite in three successive generations

Worm sex	Control	1st generation	2nd generation	3rd generation
	Mean number of recovered worms/mous	% reduction of recovered worms	% reduction of recovered worms	% reduction of recovered worms
Female	38.7	27.7	71.6	76
Male	44.6	41	75.3	70.2
Total	83.3	34.8	73.6	72.9

lethal effects (**Ducklow *et al* 1979; Cheng, 1986; El Emam *et al* 1996**). The present commercial *Bacillus* bacteria, *B. thuringiensis kurstaki*, is commenly used in Egypt as a biocontrol agent in the combat against the bollworms *Pectinophora gossypiella* and *Earias insulana* (**El-Gemeiy *et al* 1999; El-Gemeiy, 2001**). It had been tested for molluscicidal effect on *B.alexandrina* snails, specially on hatchability of eggs, survival and growth of snails by **Gamal *et al* (2000)**.

In the present work, this bacteria was used for the first time to test its effect on *S. mansoni* infection of *B. alexandrina* and on the development of the parasite. This investigation aimed also to elucidate whether this effect is extended to successive generations of the parasite in both snails and mice. Information obtained from this study should help in evaluating potential effect of this bacteria on transmission of schistosomiasis.

The results indicated that infection of snails with this bacteria in both cases showed a deleterious effect on the life cycle of the parasite by reducing the infection rate of snails, delaying the intramolluscan development as well as reducing the worm recovery in the final host (mice). This effect was clear in the three successive generations of the parasite. The present findings are different from the results of **El Emam *et al* (1996)** and **El Emam** & **Haroun, (1999)** who worked on another strain of *Bacillus* namely *B. thuringiensis israelensis*. The former authores observed that the cercarial production of infected *B. alexandrina* was suppressed to minimal after two weeks of bacterial infection, while the later authors claimed that the bacteria did not exhibit any significant effect on cercarial production and duration of cercarial shedding. The difference in results between the present and other works may be attributed to strain difference of the bacteria used in both cases, being *kuristaki* in the present work and *israelensis* in their work. *B. thuringiensis israelensis* is known to be a mosquito larvicide **(De Bargae, 1978; Bekheit, 1984**).

The possible use of bacteria as a biocontrol agent against schistosomiasis vector snails was suggested by several investigators**. Berry (1949) and Michelson (1957)** observed a disease with high degree virulence against *Australorbis glabratus, Physopsis africans* and *B. pfeifferi* snails and added that this disease was due to a gram negative bacterium. Most work on effect of bacteria on medically important snails were carried out on *Bacillus pinottii*. Thus, this bacteria was used in laboratory and field trial to control *A. glabratus* in Venzuella **(Textra & Scorza, 1954)** and in Egypt, **(Dias & Dawood, 1955)**. The latter authors added that this bacteria is harmless to vertebrates. Later on, **Ducklow *et al* (1979)** and **Cheng (1986)** recorded in wild and laboratory populations of *B. glabrata* snails several pathogenic bacterial genera.

In conclusion, the present effect of *B. thuringiensis kurstaki* on schistosomiasis transmission may add to its larvicidal potency against the cotton bollworms.

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تأثير عدوى قواقع بيومفلاريا اليكسندرينا ببكتريا باسيلس ثيورنجنسيس كيورستاكي علي ثلاثة أجيال متتالية من دودة شيستوسوما مانسوني

[37]

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درس تأثير بكتيريا Bacillus thuringiensis kurstaki علي الأطوار المختلفة لطفيلي البلهارسيا المعوية شيستوسوما مانسوني لثلاثة أجيال متتالية داخل كل من قوقع Biomphalaria alexandrina والفئران البيضاء، فقد استخدم تركيز الجرعة تحت السامة (0.08جرام/لتر ماء المحتوية علي 32000 وحدة دولية/ مجم) علي مجموعتين من القواقع: حيث عوملت المجموعة الأولي بالبكتريا بعد عدواها بميراسيديم الطفيلي مباشرة وتركت البكتريا مصاحبة للقواقع لمدة أسبوع ثم نقلت إلي مياه نظيفة. أما المجموعة الثانية من القواقع فقد عوملت بالبكتريا لمدة أسبوع قبل تعريضها للميراسيديا. وقد أوضحت نتائج المجموعة الأولي انخفاض نسبة العدوى في القواقع للأجيال الثلاثة للطفيلي حيث كانت 60 و18 و66.6% علي الترتيب، في مقابل 90% و92% و90% في القواقع غير المعاملة بالبكتريا. أما فترة حضانة الطفيلي في القوقع فقد زادت ، حيث كانت 29.1 يوما و33 يوما و38.5 يوما في المتوسط ، بينما كانت في مجموعة المقارنة 27 يوما. وقد لوحظ انخفاض أعداد ديدان الطفيلي
المستخلصة من الفئران المعدية بنسب 52% و78.4% و58.6% للأجيال الثلاثة علي الترتيب.

أما نتائج المجموعة الثانية فقد أظهرت أن نسبة العدوى في القواقع قد انخفضت إلي 40% و16% و73.7% في الأجيال الثلاثة للطفيلي علي التوالي. وزادت فترة حضانة الطفيلي داخل القواقع لتصبح 32 يوما و32 يوما و35 يوما في المتوسط علي الترتيب، بينما كانت ثابتة في مجموعة قواقع المقارنة عند 27 يوما. أما أعداد ديدان الأجيال الثلاثة للطفيلي داخل الفئران فقد أظهرت انخفاضا بنسبة 34.8% و73.6% و72.9% علي الترتيب.

وبذلك فإن هذه النتائج تثبت أن عدوى قواقع بيومفلاريا اليكسندرينا بالجرعة تحت السامة من بكتريا باسيلس ثيورنجنسيس كيورستاكي تحدث آثارا سلبية واضحة علي انتقال طفيلي شيستوسوما مانسوني لثلاثة أجيال متتالية مما يرشح هذه البكتريا لتكون عامل محتمل للمكافحة البيولوجية للبلهارسيا المعوية.

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