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

Samia N. El-Bardicy\(^1\); Menerva M. Tadros\(^1\) and Sherif M. Hafez\(^2\)

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 successive 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 suppressing 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 kurstaki (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 dechlorinated 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 dechlorinated 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 1<sup>st</sup> and 2<sup>nd</sup> generations. While the reduction was much less in the 3<sup>rd</sup> 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 1<sup>st</sup> case than the 2<sup>nd</sup> 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

<table>
<thead>
<tr>
<th>Parameter</th>
<th>First generation</th>
<th>Second generation</th>
<th>Third generation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control infected with bacteria</td>
<td>% of change</td>
<td>Control infected with bacteria</td>
</tr>
<tr>
<td>Number of exposed snails</td>
<td>25</td>
<td>25</td>
<td>50</td>
</tr>
<tr>
<td>% of Infection</td>
<td>90</td>
<td>60</td>
<td>(-33.3)</td>
</tr>
<tr>
<td>Mean prepatent period (days)</td>
<td>27</td>
<td>29.1</td>
<td>(+7.8)</td>
</tr>
<tr>
<td>SD</td>
<td>0.3</td>
<td>4.3</td>
<td>1.4</td>
</tr>
</tbody>
</table>

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

<table>
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<th>First generation</th>
<th>Second generation</th>
<th>Third generation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control infected with bacteria</td>
<td>% of change</td>
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</tr>
<tr>
<td>Number of exposed snails</td>
<td>25</td>
<td>25</td>
<td>50</td>
</tr>
<tr>
<td>% of Infection</td>
<td>90</td>
<td>40</td>
<td>(-55.5)</td>
</tr>
<tr>
<td>Mean prepatent period (days)</td>
<td>27</td>
<td>32</td>
<td>(+18.5)</td>
</tr>
<tr>
<td>SD</td>
<td>0.3</td>
<td>1</td>
<td>1.4</td>
</tr>
</tbody>
</table>
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

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

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

<table>
<thead>
<tr>
<th>Worm sex</th>
<th>Control Mean number of recovered worms/mous</th>
<th>% reduction of recovered worms</th>
<th>1st generation</th>
<th>2nd generation</th>
<th>3rd generation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>38.7</td>
<td>27.7</td>
<td>71.6</td>
<td>76</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>44.6</td>
<td>41</td>
<td>75.3</td>
<td>70.2</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>83.3</td>
<td>34.8</td>
<td>73.6</td>
<td>72.9</td>
<td></td>
</tr>
</tbody>
</table>
lethal effects (Ducklow et al 1979; Cheng, 1986; El Emam et al 1996). The present commercial Bacillus bacteria, B. thuringiensis kurstaki, is commonly 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 molluscidal 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 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 kurstaki 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 pinotti. Thus, this bacteria was used in laboratory and field trial to control A. glabratus in Venzuela (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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Infection effect on Schistosoma mansoni


Samaa Al-Baddawi, Tarek Said, Shereef Tadros, Shereef Tadros, Shrey Fakhry. (2005). تأثير بكتيريا Bacillus thuringiensisкурستاكي على الأ柘واز المختلفة لطفيلي البلهارسيا المعوية شيستوسوما مانسوني لثلاثة أجيال متتالية داخل كل من القوقع Biomphalaria alexandrina والقنور. بحقضنة 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 بنسبة 52% و78.4% و58.6% للأجيال الثلاثة على الترتيب.

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

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

تحكيم: أ.د راوية فتحى جمال
أ.د فؤاد يوسف