INSECTICIDAL EFFECT OF *BACILLUS THURINGIENSIS VAR KURSTAKI* AGAINST *HYALOMMA DROMEDARII* ON EXPERIMENTALLY INFESTED RABBITS

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Abstract

The insecticidal effect of *Bacillus thuringiensis var. Kurstaki* against *Hyalomma dromedarii* on experimentally infested rabbits was investigated. Ten New Zealand white rabbits, 1.5-2 Kg weight for each, were used. They were divided into 2 groups. Group I was the infested-sprayed group and consisted of 8 rabbits. Group II was the infested non-sprayed group and consisted of 2 rabbits. The commercial Dipel 2x was sprayed at a concentration of 20 g/L for 2 successive days. There was significant difference (p value< 0.05) from the 1st day post- 1st spraying till the 6th day post 2nd spraying. Some tick individuals on sprayed rabbits were found dead but still attached to the host by their proboscis and they were completely paralyzed, others were collected dead. Moreover, some female ticks on sprayed rabbits had reached the engorged stage as those of control group, but, others died before or after being partially fed. There was no significant difference in the time of engorgement, or in weights between female ticks in both groups.

At the same time, the effect of *Bacillus thuringiensis* was also tested *in vitro* on different stages of *H. dromedarii*. All unfed adult ticks were paralyzed 1 - 4 days post 1st spraying, sprayed egg batches were not affected and gave larvae, while all larvae died within 2 - 3 hours after spraying. Data were statistically analysed and discussed.

INTRODUCTION

Ticks are notorious as vectors of human and animal disease agents. They transmit a great variety of infectious organisms more than any other groups of blood-sucking arthropods, and worldwide are second only to mosquitoes in terms of their public health and veterinary importance. Additionally, ticks can cause severe or sometimes, deleterious effects because of their proteins injected with the saliva (*Mullen and Durden*, 2002). They also can be injurious to domestic livestock causing anemia as a result of sucking large amounts of blood. Moreover the wounds that they produce may create sites of secondary infections and diminish the value of livestock by damaging their hides (*Soulsby*, 1982).

Historically, control of ticks was accomplished with acaricides which are the most widely used means to control or minimize tick attacks. The development of acaricides resistance by ticks is a continuing concern, as well, the hazards of acaricides for environment pollution is a current problem.

For these facts, the biological control, a new approach using Bacillus *thuringiensis var Kurstaki*, for tick had been established for minimizing the dangerous side effects of acaricides. The present study was carried out to investigate the effect of *B. Thuringiensis Kurstaki* (Dipel-2x) against *Hyalomma dromedarii* on experimentally infested rabbits.

MATERIALS AND METHODS

Engorged female ticks of the species *Hyalomma dromedarii* were collected from naturally infested camels in the quarantine of Cairo abattoir before slaughter. These ticks were identified according to *Hoogstraal* (1956). Ticks were kept and maintained at the Animal Health Research Institute. Each engorged female tick was placed separately in special tick-rearing glass containers closed tightly with cotton and gauze tampon. The containers were kept in a biological incubator regulated at 28 °C and 85% relative humidity. New Zealand white rabbits (1.5-2Kg) weight for each and proved to be free from any external or internal parasites were obtained from a special breeder. After hatching of eggs, all stages of ticks were fed on ears of rabbits using ear bags to produce a tick colony for this experiment (*Abdel Gawad*, 1977). Experimental infestation was carried out on 10 rabbits. They were divided into two groups: Group I, consisted of eight rabbits and was the infested- sprayed group, while, group II consisted of two rabbits and was the infested-non-sprayed group. Ear bags were tightly fixed around one ear of each rabbit of both groups. Ten unfed adult males and females of the obtained *H. dromedarii* tick individuals, were placed in each bag. The numbers of attached ticks were recorded. *Hyalomma dromedarii* ticks on group I were sprayed, after complete attachment to the ears of rabbits (3-5 days), with the commercial *B. Thuringiensis Kurstaki* (Dipel-2x) solution (Abbott laboratories) at a concentration of 20 g /L for two successive days (*Hassanain et a.l.,* 1997). Dropped ticks were observed daily after 24 hours post-first spraying. The dropped tick stages were counted and the fallen time was recorded and compared with those of control group. In case of presence of any engorged females, they were collected, weighed and incubated till laying and hatching of eggs. As well any changes that appeared on the sprayed ticks were recorded and illustrated.

At the same time, the effect of *B. Thuringiensis* was also tested *in vitro* on different stages of *H. dromedarii*. Twenty unfed adult tick individuals (males and females) were divided equally into two clean Petri-dishes. As well, three egg batches obtained from engorged females, each in a Petri-dish and unfed larvae were sprayed with *B. Thuringiensis*. They were observed daily, and compared with non-sprayed ten adults; an egg batch and larvae, each was put in a Petri-dish. Statistical analysis was carried out according to SPSS Win. Version 5.

RESULTS

Table 1 showed the effect of *B. Thuringiensis* against *H. dromedarii* on experimentally infested rabbits from the day of complete attachment of ticks, before spraying (zero day) till the termination of experiment (6th days post- 2nd spraying). It was noted that, not all ticks applied on rabbits attached to them. From the 1st day post-1st spraying there was significance (p value <0.05) till the 6th day post-2nd spraying. Some tick individuals on sprayed rabbits were found dead but still attached to the host by their proboscis, and they were completely paralyzed (Fig. 1), others were collected dead in the ear bags. Moreover, some female ticks on sprayed rabbits had reached to the engorged stage as those of control group, but, other females on sprayed rabbits died before or after being partially fed. Each engorged female from both groups was weighed. There was no significant difference in the time of engorgement between sprayed and control groups (5-7days). As well, no differences in weights between both groups were noticed (Table 2).

Each engorged female obtained from sprayed and control groups was then incubated separately. Eight-ten days later, females began to lay their egg batches and after 37-40 days incubation live larvae were obtained.

Concerning spraying adult tick individuals, egg batches and larvae *in vitro* with *B. Thuringiensis*, and all unfed adult ticks (20) were paralyzed after 1-4 days post-1st spraying, while, the non-sprayed 10 adult ticks were still alive. However, sprayed egg batches were not affected and gave larvae as those of non- sprayed ones, while, all larvae died within 2-3 hours after spraying.

Fig. 1. H. dromedarii tick showing paralysis in left legs on rabbit ear after spraying with *Bacillus thuringiensis*.

Table 1. Effect of *B. Thuringiensis* against *H. dromedarii* on rabbits.

Days of tick attachment	No. of ticks on treated rabbits (mean + SE)	No. of ticks on control rabbits (mean + SE)	P value
Zero day	5.9 ± 0.79	9 ± 0.38	0.15
1st day post-1st spraying	4.4 ± 0.84	9 ± 0.38	0.003 *
1st day post-2nd spraying	3.5 ± 0.78	8.5 ± 0.19	0.001 *
2nd day post-2nd spraying	3.4 ± 0.73	8 ± 0.00	0.000 *
3rd day post-2nd spraying	3 ±0.68	7 ± 0.19	0.001 *
4th day post-2nd spraying	2.6 ± 0.71	6.5 ± 0.57	0.006 *
5th day post-2nd spraying	2.3 ± 0.80	6 ± 0.38	0.005 *
6th day post-2nd spraying	1.9 ± 0.83	6 ± 0.38	0.003 *

SE = standard error

*Significance is at p value < 0.05

Table 2. Number of engorged females obtained in both treated and control groups and their weights.

Number and weights of engorged females	Treated group								Control group	
	1	2	3	4	5	6	7	8	1	2
No.of attached ticks on zero day	7/10	7/10	5/10	3/10	9/10	8/10	5/10	3/10	10/10	8/10
No. of engorged females from attached ticks	0	3	1	0	3	1	0	0	4	1
Weight of engorged females /g	-	1.03 0.70 0.50	1.09	-	0.71 0.70	0.70	-	-	0.85 0.80 0.72 0.75	0.66

DISCUSSION

The present study was carried out to investigate the insecticidal effect of *B. thuringiensis var Kurstaki* (Dipel 2x) against *H.dromedarii* on experimentally infested rabbits. The control of ticks and tick-borne diseases usually was accomplished with acaricides, which were the most widely used means to control or minimize tick attacks. However, the development of acaricides resistance by ticks was a continuing concern (Mullen *and durden*, 2002). Recently, the biological control methods *with B. thuringiensis,* had become a new approach and easy method to overcome such resistance. *Bacillus thuringiensis* is a naturally occurring soil bacterium. It is considered an ideal mean for pest management because of its specificity to pests and its lack of toxicity to human and animals. *Pearce et al.,* (2002) proved that there was no harmful effect on children with asthma living in zones sprayed with *Bacillus thuringiensis Kurstaki*. As well, there was no residue of Dipel 2x in brain, liver, kidney, spleen and lungs of rats after oral administration at a dose of 50mg/100g body weight for 7 successive days when its antibacterial effect against aerobic and anaerobic bacteria was tested (*Sobhy et al., 2000*). Also, *Bacillus thuringiensis* was successfully used against mosquitos (*Rashed and Teleb, 1998*) and *Trichostrongylidae* parasites (*Abdel-Rahman et al., 1998*).

To be effective, insect must eat *Bacillus thuringiensis* during their feeding stage of development. This Bacillus forms spores, which contain unique crystalline bodies. These crystals are composed of proteins known as delta-endotoxins; when eaten, the spores and crystals act as poisons in insects. Therefore, *Bacillus thuringiensis* is referred to be a stomach poison, as such crystals dissolve in the intestine causing paralysis of cells in the gut. The spores can also invade other insect tissue and multiply in the insect's blood untill the insect dies within a few hours to a few weeks, depending on the amount of *Bacillus thuringiensis* ingested (http:// extoxnet. Orst. Edu /pips/ bacillus. Htm). *Abdel-Megeed et al*, (1997) found changes in hemolymph components of *Argas persicus* after spraying with *B. Thuringiensis* directly, such changes were not observed in *Argas* infested hens injected subcutaneously with different concentrations of Dipel 2x. As well, *Hassanain et al* (1997), had isolated *B. Thuringiensis* from the hemolymph of *H. dromedarii* 40 hours post-spraying. *Abdel Megeed et al* (1999), sprayed *Boophilus annulatus* infested calves with Dipel 2x, hard ticks died six days post-spraying.

In the present study, it was noticed that there was a variety of tick attachment to the ears of experimentally infested rabbits; this might be due to the host susceptibility. After spraying *H. dromedarii* developmental stages on experimentally infested rabbits with *B. thuringiensus*, there was a significant difference with control group (p value < 0.05) till the termination of experiment. Some tick individuals were found paralyzed, but, were still attached with their proboscis to the animals. This fact was in agreement with *Abdel-Megeed et al.* (1997), who observed that dead *Boophilus annulatus* were still found in sites of infested spraying calves with *B. Thuringiensis*. *Mullen and Durden* (2002), stated that, the attachment of ticks was reinforced by secretion of cement substances with the saliva into and around the wound site, so, sprayed ticks were still found on rabbits although they were dead due to such cement materials. However, some female ticks had reached to the engorged stage and laid eggs which had hatched afterwords.

Studying the effect of *B. Thuringiensis in vitro* was compared to that *in vivo.* All adult tick individuals succumbed 1-4 days post-spraying, all larvae died 2-3 hours post-spraying, but, eggs were not affected. *Zhioua et a.l.* (1999), showed that engorged larvae of *Ixodes scapularis* when dipped in solution of *B. Thuriniensis var Kurstaki* showed 96% mortality 3 weeks after dipping. Regarding eggs, *Mullar and Durden* (2002) stated that the emerging eggs were waxed by Genes organ, such wax might prevent the penetration of Dipel 2x sprayed into the eggs, so such eggs became still unaffected and hatched.

The remarkable and rapid death of adult ticks after spraying *in vitro* might be referred to the longer exposure of ticks in the Petri-dishes; such ticks were soaked for a time in *B. Thuringiensis* after spraying. *Abdel-Rahman and Hassanain* (1997) stated that, the exposure time was an important factor upon which Dipel 2x effect was based. This effect was observed on *Lymneae caillaudi* snails, as time was necessary for toxins or other active secreted components to cause damage or to reach to its optimal concentrations essential for this damage. *Hassanain et a.l* (1997) found that *Argas persicus* died 36 hours- 5 days, while, *Hyalomma dromedarii* died 48 hours and 10 days post-treatment with *B. Thuringiensis in vitro.*

From the present study, it was concluded that, it was more effective to spray *B. thuringiensis var Kurstaki* directly on ticks than on infested animals. As all adult tick individuals and all larvae succumbed post-spraying with *B. thuringiensis* in vitro, while spraying ticks on infested rabbits with *B. thuringiensis* showed that some adult ones had reached to the engorged stage. So, the spray of Dipel 2x could be effectively used to control ticks found in animal houses.

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التأثير الحشري القاتل لبكتيريا باسيلس ثيرنجينسيس كورستاكي ضد هيالوما درومیداري على الأرانب المعدية تجريبيا

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تم دراسة التأثير الحشري القاتل لبكتيريا باسيلس ثيرنجينسس ضد هيالوما درومیداري علـــى الأرانب المعدية تجريبيا. وقد تم استخدام ١٠ أرانب نيوزيلندي بيضاء يزن الواحد ٥, ١–٢كجم، وتم تقسيمها إلى مجموعتين: المجموعة الأولى تتكون من ٨ أرانب وهي المجموعة التي تمـت عـدواها بالقراد ثم رشها، أما المجموعة الثانية فتتكون من أرنبين وهي المجموعة التي تم عدواها ولم ترش. وقد تم رش البكتريا بتركيز ٢٠ جرام/ لتر لمدة يومين متتاليين. وبعد الرش، تبين ان هنـاك فرقـاً معنويا من اليوم الأول بعد الرش الأول إلى اليوم السادس بعد الرش الثاني. وقد وجد بعـض أفـراد القراد في المجموعة الأولى ميتة ولكنها مازالت عالقة بالأرانب عن طريق الفم ومصابة بالشلل التام، بينما تم تجميع البعض الأخر ميتا، كما وجد أيضا بعض إناث القراد في المجموعـة الأولـى وقـد وصلت إلى الطور كامل التغذية كما في المجموعة الضابطة لكن البعض الأخر مات قبل أو بعـد أن أصبح في طور نصف مغذي. وقد ثبت إحصائيا انه ليس هناك فرق معنوي بين مدة الوصول إلـى الطور كامل التغذية أو في الأوزان بين أنثى القراد في كلتى المجموعتين.

في نفس الوقت تم دراسة تأثير البكتيريا على الأطوار المختلفة لقراد هيالوما درومیداري معمليـا، ووجد أنه قد حدث شلل تام بعد رش الأطوار البالغة قبل التغذية بعد ١–٤ أيام من الرش الأول، كما أنه لم يحدث أي تأثير لمجموعات البيض بعد الرش وتم الفقس ونتجت يرقات، في حين انه عندما تم رش اليرقات ماتت بعد ٢–٣ ساعات من الرش وقد تم تحليل البيانات إحصائيا ومناقشتها.