RESEARCH ARTICLE

Off-host Study on Use of Bacterial Biocontrol Agents against Ruminant Tick *Rhipicephalus microplus* under Field Conditions

Balaji S. Jadhav[1], Babasaheb W. Narladkar[2], Jagdish G. Gudewar[3], Gajanan M. Chigure[4], Gajendra N. Bhangale[5]*

Abstract

In an off-host study, bacterial biocontrol agents, *viz., Bacillus thurinngiensis var kurstaki, Bacillus thuringiensis* var *kurstaki* toxin (Dipel 8L), *Bacillus thuringiensis var israelensis, Bacillus weihenstephanensis var WSBC* and *Bacillus weihenstephanensis var KBAB4* were evaluated against *Rhipicephalus* (*Boophilus*) *microplus* ticks. All four bacteria and one toxin were evaluated at five different concentrations. The LC_{50} values of one toxin and three bacteria, viz., *Bacillus thuriengiensis var krustakivar* (Dipel 8L) toxin, *Bacillus thuriengiensis var kurstaki, Bacillus thuriengiensis var isaralensis* and *Bacillus weihenstephanensis var WSBC* in mg/liter were 0.009 and 0.009 (5544 IU each); 0.001 and 0.001 (616 IU each); 0.001 and 0.080 ($2.0x10^7$ and 1.6×10^6 spores); and 0.001 and 0.001 (1.3×10^7 spores) against adult ticks and eggs, respectively. The value of LC_{50} against egg stage for *Bacillus weihenstephsnensis var KBAB4* was 0.001 mg/liter, and it could not be determined for adult ticks. When LC_{50} values of these bacterial biocontrol agents were compared with their LD_{50} values from literature in rats, these bacteria and toxin appeared to be safe for field conditions.

Keywords: Bacterial BCA, *Bacillus thuringiensis,* Ruminant ticks, LC_{50}.

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Introduction

Ticks are obligate, blood-sucking ectoparasites of vertebrates, particularly mammals and birds (Wall and Shearer, 2008). Because of its one-host life cycle, all stages of development occur on the same host. Ticks transmit a greater variety of infectious organisms than any other group of arthropods. Worldwide, they are second only to mosquitoes in terms of their public health and veterinary importance. Ruminant tick *Rhipicephalus microplus* (formerly *Boophilus microplus*) is considered the most important tick parasite of livestock in subtropical and tropical regions. It is also endemic in the Indian region. *R. microplus* is, a hard tick found on various hosts, including domestic and some wild animals, causes heavy economic losses (Ghosh and Nagar, 2014; Narladkar, 2018) due to babesiosis and theileriosis two economically important tick-borne diseases of livestock (Rao *et al.,* 2018).

Chemical acaricides like organophosphates, synthetic pyrethroids, formamidines and macrocylic lactones have been the major tool for tick control in India. Initially almost all the commercial formulations showed very high efficacy, but indiscriminate and long-term use of these drugs sooner led to the development of resistance, resulting in decreased efficacy and treatment failure. These ultimately allowed the scientific fraternity to tap the unconventional tools for control of these pests such as phyto-compounds, integrated management and other biocontrol agents (BCAs). Several studies have been conducted to evaluate the efficacy of fungal agents, which are naturally detrimental to the growth of these ticks (Ostfeld *et al.,* 2006) and also some of bacterial agents have been reported to have predatorial effects on acarids (Machado-Ferreira *et al.,* 2015). Jouzani *et al.* (2017) studied the *Bacillus thuringiensis* as a successful insecticide with new environmental features and tidings. Due to the availability of new scientific and molecular biotechnologies, some other new potentials of Bt have recently been explored. Thus, this study was conducted to explore Bt's bacterial biocontrol agents against wide spread tick species *R. microplus*.

[1-4]Department of Parasitology, College of Veterinary and Animal Sciences, Parbhani, Maharashtra Animal and Fisheries Science University, Nagpur, India.

Corresponding Author: Gajendra Namdeo Bhangale, Department of Parasitology, College of Veterinary and Animal Sciences, Parbhani, Maharashtra Animal and Fisheries Science University, Nagpur, India., e-mail: gajumanu@gmail.com

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Materials and Methods

The work was undertaken in the Department of Veterinary Parasitology of the College at Parbhani, India. Off-host trials were undertaken in the cattle sheds located in nearby village Raipur. The bacterial powders/cultures of *Bacillus thuringiensis var israelensis, Bacillus weihenstephanensis var WSBC,* and *Bacillus weihenstephanensis var KBAB4* in the lyophilized form available in the Department were isolated during the research project on IPM concluded during the year 2012. However, *Bacillus thurinngiensis var kurstaki* bacterial culture and *Bacillus thuringiensis* var *kurstaki* toxin were obtained from authorized insecticide store in Parbhani with the trade name Dipel-8L (Valent Biosciences corporation, USA). All these agents were used to compare their efficacy against *R. microplus* ticks and their eggs as given in Table 1.

Methodology Adapted for Field Trials

The bacterial candidates were sprayed on eggs and adult stages of *Rhipicephalus microplus* ticks under field conditions in the cattle sheds - the most favorable breeding site of ticks (*off-host* studies) as per Narladkar *et al.* (2015). In each shed, only one bacterium was tested. For each bacterium six sites were marked and eggs and ticks from each site were studied for further observations. In all for five bacterial agents five different sheds were included in the study. In each shed, six sites under cracks and crevices and mangers were marked, where recently fallen blood-engorged female ticks and laid eggs population were observed. The prepared bacterial concentration (as per Table 1) was sprayed @ 30 ml per square meter area at each site.

Collection of Eggs and Adult Stages from Spraying Sites

After spraying of bacterial solutions, eggs and adult female ticks were collected on day 3, 7, and 14 post-treatment. A requisite number of female ticks were collected from cattle (cows/bullock) shed sites chosen using forceps and were identified using zoom stereoscopic microscope by referring the keys provided by Walker *et al.* (2003).

Criteria for Assessment of Efficacy of Bacterial Biocontrol Agents (BBCAs)

The efficacy of BBCAs was evaluated on the basis of mortality of adult ticks and eggs, reduction in egg laying capacity, hatchability of eggs laid by treated female ticks, and hatchability of treated eggs of *R. microplus* ticks. The test procedures described by Srivastava *et al.* (2008) and Narladkar and Shivpuje (2015) were followed with little modification. Mortality data was tabulated and efficacy was worked out in terms of per cent mortality. Similarly, to judge the egg laying capacity, ticks were observed for a number of eggs laid and compared with control ticks collected from the same shed as a place where only water was spread and considered untreated control.

For collection of eggs from treated females, the field-treated live females of *R. microplus* ticks were separately maintained. Eggs collected from such female ticks were

Table 1: LC_{50} values for different subspecies of bacteria evaluated against adult and egg stage of *Rhipicephalus microplus* and literature based LD_{50} values for acute oral toxicity in rats

Sr. No	*Bacteria*	*Ticks (LC_{50} value)*		*LD_{50} value for acute oral toxicity in rats*	*References*
		Adults	*Eggs*		
1	*Bacillus thuringiensis var israelensis*	LC_{50}=0.001 mg/litre Each gram powder contains 2.0x10^{10} Spores LC_{50}=2.0×10^{7} spores	LC_{50}=0.080 mg/litre Each gram powder contains 2.0x10^{10} Spores LC_{50} =1.6×10^{6} spores	Less than 50 mg/kg	www.planet natural.com Last accessed 06/07/2018. Rats exposed dermally (LD50>2000 mg/kg, [4.6x10^{10} CFU/kg] (McClintock *et al.,* 1995). *Bti* is proven effective and has low levels of toxicity to humans and wildlife, with minimal effect on non-target species (Hicks, 2001).
2	*Bacillus weihenstephanensis* var (WSBC)	LC_{50}=0.001 mg/litre Each gram powder contains 1.3x10^{10} Spores LC_{50}=1.3×10^{7} spores	LC_{50}=0.001 mg/litre Each gram powder contains 1.3x10^{10} Spores LC_{50}=1.3×10^{7} spores	Not known	--
3	*Bacillus weihenstephanensis* var (KBAB4)	--	LC_{50}=0.001 mg/litre Each gram powder contains 1.3x10^{10} Spores LC_{50}=1.3×10^{7}spores	Not known	--
4	*Bacillus thurinngiensisvar kurstaki*	LC_{50}=0.001 ml/litre 1ml =35000 ppm =616000 IU LC_{50}=616 IU	LC_{50}=0.001 ml/litre 1ml =35000 ppm =616000 IU LC_{50}=616 IU	6.7×10^{11} spores 5000 mg/kg	Ahmed *et al.* 2015
5	*Bacillus thuringiensis* var *kurstaki* (toxin)	LC_{50}=0.009 mg/litre 1ml =35000 ppm =616000 IU LC_{50}=5544 IU	LC_{50}=0.009 mg/litre 1ml =35000 ppm =616000 IU LC_{50}=5544 IU	a) 5 ng b) 500 ng	Tabanshik *et al.* (1992).

counted in petri dishes in batches of 100 numbers. Similarly, field-treated eggs of R. microplus were collected and counted in petri dishes in 100 numbers. Eggs were transferred in tubes which were closed with a piece of muslin cloth tied with rubber band. These tubes were maintained in desiccators at 75 % RH. The eggs were observed for hatching till the hatching process of eggs in the control group was completed.

Determination of LC_{50} Values

LC_{50} values were worked out by using the software downloaded from https://www. aatbio.com/tppls/LC_{50} calculator and compared with LD_{50} values of acute toxicity in rats referred from literature for the same bio-control agents.

Results and Discussion

The LC_{50} values of four bacteria and one toxin against eggs and adult stages of cattle tick *Rhipicephalus microplus* are presented in Table 1. The values of LC_{50} in mg/liter worked out after the field trials were 0.009 and 0.009 (5544 IU each); 0.001 and 0.001 (616 IU each); 0.001 and 0.080 (2.0×10^7 and 1.6×10^6 spores); and 0.001 and 0.001 (1.3×10^7 spores) against adult ticks and eggs, respectively, for *Bacillus thuriengiensis var krustaki* (Dipel 8L) toxin, *Bacillus thuriengiensis var kurstaki, Bacillus thuriengiensis var isaralensis* and *Bacillus weihenstephanensis* WSBC. The value of LC_{50} against egg stage for *Bacillus weihenstephsnensis var KBAB4* was 0.001 mg/liter (1.3×10^7 spores), and it could not be determined for adult ticks. According to deBarjac and Coz (1979), LC_{50} values for all the Bti, Btk and Btk toxin species against egg and adult stages of *R. microplus* ticks tested were found between 4×10^3 and 4×10^4 viable spores/ml which also corroborated with findings of current study. However, the present values were quite lower than those reported by Solanke and Narladkar (2018) against same bioagents. The LD50 values of some of these bioagents tested on rats by some workers are also depicted in Table 1.

In the environment, numerous bacteria, fungi, spiders, ants, beetles, rodents, birds, and other living things contribute significantly toward limiting tick populations (Samish *et al.,* 2004). A particularly large number of bacterial flora abundantly found on ticks' body when they are on ground, and these bacteria may be benign in nature, but all cannot be explored as bio-control agents (Ali *et al.,* 1986). When ticks are on host body, they also ingest bacteria with the blood of their hosts or become contaminated from their skin. However, on host only 1.6% of unfed adult ticks and 9.0% of recently fed ticks were found infected with bacteria (Ali *et al.,* 1986).

Different bacterial species belonging to genus *Bacillus,* proved safe to human and other vertebrates, were explored and developed as BCAs. Earlier, immersion of female *Boophilus microplus* ticks in *Cedecea lapagei* suspension was reported to cause 95-100% tick mortality (Brum *et al.,* 1991). Since only spraying of bacteria was done in cattle shed in the current study, there may be inadequate concentration to achieve the lethal effect on tick life stages. The observations of current study substantiated earlier report by Brum *et al.* (1991) that a higher concentration is required for achieving the desired mortality in adult ticks.

The use of *B. thuringiensis* for cattle tick control has been reported by Ostfeld *et al.,* (2006). Recently, Solanke and Narladkar (2018) in their *in vitro* trials with *Bacillus thuringiensis var israelensis, Bacillus weihenstephanensis* var *WSBC, Bacillus weihenstephanensis* var *KBAB4*, *Bacillus sphaericus, Bacillus thuringiensis var kurstaki* and *Bacillus thuringiensis* var *kurstaki* (toxin) on *R. microplus* in laboratory conditions reported the significant reduction in egg laying capacity of female ticks, reduced hatchability of eggs laid by treated females and reduced hatchability of treated eggs. In their experiment, the LC_{50} values obtained for above 6 BCAs after evaluation were 3.5×10^5 and 3.8×10^7; 5.0×10^9 and 4.7×10^6; 2.2×10^7 and 5.6×10^5; 2.8×10^5 and 2.0×10^4 spores; 80080 IU and 55440 IU; 135520 IU and 17.430 IU, respectively, for *R. microplus* adults and eggs. All these five bacteria and one toxin have been reported efficacious acaricide with both adulticidal and ovicidal potential.

In the present study, based on LC_{50} values of one toxin and two bacteria, *viz., Bacillus thuriengiensis var krustaki var* (Dipel 8L) toxin, *Bacillus thuriengiensis var kurstaki, Bacillus thuriengiensis var isaralensis* against eggs and adult stages of cattle tick *R. microplus* compared with their LD_{50} values in rats, appeared safe for field conditions. Farmers routinely use these against crop pests and in mosquito control program on mass scale across the country, therefore without any toxicity studies, these can be used under field conditions in the integrated tick management program. Therefore, LD50 values of two bacteria, viz., Bacillus weihenstephanensis WSBC, and Bacillus weihenstephsnensis var KBAB4 are not available in the literature, even though they exerted marginal effect against cattle tick *R. microplus,* are recommended for field application through the present study. However, further detailed studies on these bacteria will unfold their potential for field use.

Conclusion

Present study evaluated efficacy of bacterial biocontrol agents in animal sheds and it was found effective in controlling the ticks off-host. Therefore it can be concluded that the bacterial biocontrol agents, *viz., Bacillus thuriengiensis var krustakivar* (Dipel 8L) toxin, *Bacillus thuriengiensis var kurstaki, Bacillus thuriengiensis var isaralensis* need to be further evaluated for its environmental suitability and toxicity.

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