Response of unfed stages of *Rhipicephalus sanguineus* to subtle variations in the composition of assembly pheromone

Dhivya Bhoopathy¹ · Bhaskaran Ravi Latha¹ · Chirukandoth Sreekumar² · V. Leela³

Received: 30 October 2014 / Accepted: 1 June 2015 / Published online: 23 June 2015
© Indian Society for Parasitology 2015

Abstract Two combinations of assembly pheromone (AP), with and without hematin were utilized as a lure for the unfed larvae, nymph and adults of *Rhipicephalus sanguineus* ticks. In-vitro trials were carried out with the AP encapsulated in calcium alginate beads and the response of different stages of ticks were recorded. Analysis of results revealed that rapid attraction was evident in unfed larvae exposed to beads containing AP without hematin. In case of unfed nymphal and adult stages, the presence or absence of hematin did not have any impact on arrestment.

Keywords Assembly pheromone · Hematin · Dog tick · Calcium alginate beads

Introduction

Arthropods are “remarkable chemists” as they have evolved an incredible cornucopia of compounds to deal with the need to communicate information (Sonenshine et al. 1986). Such information-bearing/signalling compounds or chemicals are termed as “semiochemicals”. These semiochemicals encompasses compounds which are intraspecific (pheromones) and interspecific (allomones and kairomone) in nature. In ticks, there are three major categories of

pheromones (Hamilton 1992) namely, sex pheromones, assembly pheromones (APs) and attraction-aggregation-attachment pheromone. Of these the AP is known to be important for survival as it prevents desiccation of ticks by arresting them in favourable microenvironments (Sonenshine 2004). Components of tick faeces- adenine, guanine, xanthine and hematin are the primary constituents of the AP of ticks. A defined ratio of these compounds is known to elicit response 100 times more effectively than each of these compounds presented alone (Sonenshine 2004).

In the current study, attempts were made to prepare slow-release micro particles of AP. As the AP is a mixture of four compounds, major constraint encountered during the process of preparing slow release formulations was the relative variations in the chemical properties of each component of the AP. Hematin hampered the process of encapsulation by dissociating into the cross-linking agents. As subtle variations in the composition of pheromones can change the response of ticks the possibility of excluding hematin was questionable. Hence the present study was envisaged to ascertain the role of hematin in arrestment of different unfed stages of brown dog tick. Two compositions of AP, namely, with and without hematin were evaluated and the responses of different stages of brown dog tick, *Rhipicephalus sanguineus* to these combinations were recorded.

✉ Dhivya Bhoopathy
dhivvet@gmail.com

¹ Department of Veterinary Parasitology, Madras Veterinary College, Chennai 600 007, India

² Post Graduate Research Institute in Animal Sciences, Kattupakkam, Kanchipuram 603 203, India

³ Department of Veterinary Physiology, Madras Veterinary College, Chennai 600 007, India

Materials and methods

Ticks for in vitro bioassay

The brown dog tick, *R. sanguineus* was collected by visual appraisal from the infested dogs presented at the Small Animal Clinics, Madras Veterinary College Teaching

Hospital, Chennai and from dogs housed at Blue Cross of India, Velachery. Engorged females were maintained in trays filled with moist sand to maintain a relative humidity of >85 % for it to oviposit and the egg to hatch into larvae. Hungry larvae were utilized soon after hatch. Engorged larvae and engorged nymphs were collected and maintained in a similar manner until they hatched out to nymph and adults respectively. The hungry nymph and adult ticks were used soon after hatch in the in vitro bioassays.

Assembly pheromone

The synthetic analogues of AP viz., adenine, guanine, xanthine and hematin were used (Sigma-Aldrich, USA). These were used in the ratio indicated by Sonenshine (2004) and Ranju (2011). The response of ticks to two different combinations of AP—with and without hematin was studied. The composition of AP with hematin was as follows:

Guanine	95 mg
Xanthine	3.8 mg
Adenine	3.8 mg
Hematin	3.8 mg
Total	106.4 mg

The composition of AP without hematin was as follows:

Guanine	95 mg
Xanthine	3.8 mg
Adenine	3.8 mg
Total	102.6 mg

Preparation of calcium alginate beads

The calcium alginate beads were prepared as per the procedure of Anwar et al. (2009). Plain beads without AP were

also tested in in vitro assays to rule out response of ticks to the polymer. The size of the particles was assessed using scanning electron microscope.

In-vitro evaluation of calcium alginate beads by petridish bioassay

A modified method of Yoder and Stevens (2000) as adopted by (Ranju et al. 2012) was employed in the current study. Glass petridish of 20 cm diameter were used for the assay. The AP encapsulated calcium alginate beads were placed in one quadrant of the petridish. Unfed larvae, nymph and adult stages were placed in the opposite quadrant. All tests were conducted at room temperature and results read after 10 min, 30 min, 2 h and 24 h. Tests were replicated with N = 500 for unfed larvae, N = 50 for unfed nymph and N = 100 for unfed adults for each combinations with and without hematin and polymer control.

Statistical analysis

Statistical analysis was done using Chi square test.

Results

A cascade of behavioural responses of the different unfed stages of *R. sanguineus* ticks on exposure to AP encapsulated within calcium alginate beads included momentary questing, assumption of feeding posture (lowered palps and raised posterior part) and cessation of ambulatory activity resulting in formation of clusters. No such behavioural responses were evident when the ticks were exposed to polymer control beads.

The response of unfed larval, nymphal and adult stages to beads with and without hematin during various time frames is shown in Tables 1, 2 and 3. On analysis of the response of ticks, the beads without hematin elicited much

Table 1 Response of unfed larvae of *R. sanguineus* to assembly pheromone encapsulated in calcium alginate beads

Calcium alginate beads	Response of larvae 10 min post-exposure		Response of larvae 30 min post-exposure		Response of larvae 2 h post-exposure		Response of larvae 24 h post-exposure	
	Active	Akinetic	Active	Akinetic	Active	Akinetic	Active	Akinetic
Polymer control	500	–	500	–	500	–	500	–
Assembly pheromone with hematin	468 (93.6)	32 (6.4)	425 (85)	75 (15)	380 (76)	120 (24)	360 (72)	140 (28)
Assembly pheromone without hematin	448 (89.6)	52 (10.4)	399 (79.8)	101 (20.2)	341 (68.2)	159 (31.8)	279 (55.8)	221 (44.2)
χ^2 value	5.20*		4.66*		7.56**		28.44**	

Figure in the parenthesis indicates percentage

* Significant ($p < 0.05$), ** Highly significant ($p < 0.01$)

Table 2 Response of unfed nymphs of *R. sanguineus* to assembly pheromone encapsulated in calcium alginate beads

Calcium alginate beads	Response of nymphs 10 min post-exposure		Response of nymphs 30 min post-exposure		Response of nymphs 2 h post-exposure		Response of nymphs 24 h post-exposure	
	Active	Akinetic	Active	Akinetic	Active	Akinetic	Active	Akinetic
Polymer control	50	–	50	–	50	–	50	–
Assembly pheromone with hematin	47 (94)	3 (6)	40 (80)	10 (20)	32 (64)	18 (36)	25 (50)	25 (50)
Assembly pheromone without hematin	45 (90)	5 (10)	37 (74)	13 (26)	30 (60)	20 (40)	22 (44)	28 (56)
χ^2 value	0.54 ^{NS}		0.51 ^{NS}		0.17 ^{NS}		0.36 ^{NS}	

Figure in the parenthesis indicates percentage

NS non-significant ($p > 0.05$)

Table 3 Response of unfed adults of *R. sanguineus* to assembly pheromone encapsulated in calcium alginate beads

Calcium alginate beads	Response of adults 10 min post-exposure		Response of adults 30 min post-exposure		Response of adults 2 h post-exposure		Response of adults 24 h post-exposure	
	Active	Akinetic	Active	Akinetic	Active	Akinetic	Active	Akinetic
Polymer control	100	–	100	–	100	–	100	–
Assembly pheromone with hematin	73 (73)	27 (27)	45 (45)	55 (55)	40 (40)	60 (60)	30 (30)	70 (70)
Assembly pheromone without hematin	75 (75)	25 (25)	56 (56)	44 (44)	39 (39)	61 (61)	28 (28)	72 (72)
χ^2 value	0.10 ^{NS}		2.42 ^{NS}		0.02 ^{NS}		0.10 ^{NS}	

Figure in the parenthesis indicates percentage

NS non-significant ($p > 0.05$)

rapid response in the larvae. The statistical analysis also confirmed this finding to be significant throughout the observation period. In case of unfed nymphal and adult stages, the presence or absence of hematin did not have any impact on arrestment.

Discussion

In the present study, trials using calcium alginate beads with and without hematin revealed that hematin did not play a stellar role in arrestment of unfed nymphal and adult stages of *R. sanguineus* ticks. The finding was contrary to that recorded in *Ixodes scapularis* wherein hematin was found necessary for assembly of unfed adults (Sonenshine et al. 2003). Inter-specific variations of AP and change in sensitivity of ticks to minor changes in the composition of pheromones could be responsible for this (Sonenshine 1985). The response of larvae was substantially lower when exposed to calcium alginate beads containing AP with hematin revealing that the absence of hematin in the beads definitely influenced the outcome of the experiment. Sonenshine (1985) has opined that substantial differences occur in the assembly response of different life stages and physiological states whether fed or unfed which may be the

reason for the differences in the response of larvae compared to that of nymph and adult. In conclusion, it can be inferred that role of hematin was negligible in the arrestment of nymphal and adult stages while absence of hematin enhanced the arrestment of the unfed larval stages.

Acknowledgments The first author expresses her sincere gratitude to the Department of Science and Technology, New Delhi for financially assisting her doctoral research by providing her with INSPIRE fellowship.

References

- Anwar A, Qader SAU, Riaz A, Iqbal S, Azhar A (2009) Calcium alginate: a support material for immobilization of proteases from newly isolated strain of *Bacillus subtilis* KIBGE-HAS. World Appl Sci J 7(10):1281–1289
- Hamilton JGC (1992) The role of Pheromones in tick biology. Parasitol Today 8(4):130–133
- Ranju RS (2011) Application of semiochemicals for the control of Ixodid ticks. M.V.Sc., thesis. Submitted to Tamil Nadu Veterinary and Animal Sciences University, Chennai
- Ranju RS, Latha BR, Leela V, Basith SA (2012) Effect of 1-octen-3-ol, the ruminant kairomone on ixodid tick larvae. Vet arhiv 82(6):609–615
- Sonenshine DE (1985) Pheromones and other semiochemicals of the Acari. Ann Rev Entomol 30:1–28
- Sonenshine DE (2004) Pheromones and other semiochemicals of ticks and their use in tick control. Parasitol 129:S404–S425

- Sonenshine DE, Taylor D, Carson KA (1986) Chemically mediated behaviour in acari. *J Chem Ecol* 12(5):1091–1108
- Sonenshine DE, Adams T, Allan SA, McLaughlin J, Webster FX (2003) Chemical composition of some components of the arrestment pheromones of black-legged tick, *Ixodes scapularis* (Acari: Ixodidae) and their use in tick control. *J Med Entomol* 40(6):850–859
- Yoder JA, Stevens BW (2000) Attraction of immature stages of the American dog tick (*Dermacentor variabilis*) to 2, 6-dichlorophenol. *Expl Appl Acarol* 24:159–164