The Journal of Zoology Studies
We Don't Love Animals, We Live For Them
www.journalofzoology.com

ISSN 2348-5914
JOZS 2014; 1(3): 14-18
JOZS © 2014
Received: 03-05-2014
Accepted: 16-05-2014

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Effect of ruminant kairomone volatile fatty acids on larval stages of five ixodid tick species

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Abstract
In vitro evaluations were made to assess the effect of volatile fatty acids namely butyric acid, propionic acid and a combination of both on the larval stages of all five ixodid tick species which are prevalent in Tamil Nadu namely *Rhipicephalus(Boophilus) microplus, Haemaphysalis bispinosa, Rhipicephalus haemaphysaloides*, *Hyalomma marginatum* and R*hipicephalus sanguineus* . The study revealed that *H. marginatum* and *R.haemaphysaloides* showed maximum attraction to butyric acid (43 %) where as *H. bispinosa* was least attracted (32 %). With propionic acid *H. marginatum* revealed highest attraction (43 %) followed by *R. haemaphysaloides* (40 %). The least attraction towards propionic acid was exhibited by *R. microplus* larval stages (28 %). Combination of propionic acid and butyric acid resulted in maximum attraction of 46 per cent among *H. marginatum* followed by *R. microplus* larvae (43 %). The least attraction to the combination trial was exhibited by *H. bispinosa* larvae (22 %). *Rhipicephalus sanguineus* larvae did not exhibit any attraction or demonstrate any behavioural response towards the volatile fatty acids.

Keywords: Butyric acid, Propionic acid, Ixodid tick, Ruminant kairomones, Volatile fatty acids.

1. Introduction
The impact of ticks and tick-borne diseases on the livelihood of resource poor farming communities have been ranked high[1] . These problems are closely associated with domestic animals and pets in the tropical and subtropical regions of the world. In India, the economic losses due to tick and tick-borne diseases are estimated to be US$ 498.7 million per annum [2] with a global annual loss of $109 billion [3] . In Tamil Nadu, *Rhipicephalus sanguineus* is the most common ixodid tick parasitizing dogs, while *Haemaphysalis bispinosa, Rhipicephalus haemaphysaloides* and *Hyalomma marginatum* are seen infesting small and large ruminants in addition to *Rhipicephalus microplus (Boophilus microplus*) infestation on large ruminants. Currently the mainstay of tick control measure relies mainly on the use of chemical acaricides. However the use of acaricides is often accompanied by serious drawbacks such as chemical pollution of the food chain and environment, apart from the worrisome selection of acaricide resistant ticks. An effective alternative tick control method used in other countries includes semiochemicals.

Locating a host for blood meal is one of the most formidable challenges for ticks [4]. The questing behaviour of ixodid ticks enable for identification and localisation of approaching hosts and these are evoked by chemical cues known as kairomones [5]. These chemical signal vehicles can be utilised for attracting the ticks to one location. The most notable host cue is carbon dioxide produced by host respiration. Additional cues that guide ticks to host include water vapour emitted via host respiration and evaporative losses, lactic acid present on skin, mammalian skin lipid, squalene, nitrogen containing host excretory secretory products, ammonia and urea and the volatile fatty acids emanating from ruminants [6]. Ruminants regularly eruct gases from the foregut to relieve excess pressure and maintain a chemical equilibrium, which inadvertently signal their presence to hard ticks. Rumen fluid odour is the product of a stable bioreactor whose major chemical constituents do not vary between ruminant species. Behavioural responses were recorded to acetic, propionic and isobutanoic acids which simulate rumen fluid odour [7, 8]. Volatiles attracted ticks on their own, and mixtures of these volatiles attracted ticks based on the rumen composition of volatiles. The use of entrained rumen fluid odour attracted 42 % of *A. variegatum* ticks. Single volatile fatty acid components like butyric acid and propionic acid at a concentration of 10 ng were found to elicit 34 % and 24 % attractions respectively [5]. This paper explores the effect of ruminant kairomone volatile fatty acids on the five larval ixodid ticks of Indian sub continent.

2. Materials and Methods

2.1. Ticks

Ticks were located by visual appraisal and by running the hand across the body of the animal. Dog ticks, *R. sanguineus* were collected from the animals presented in the Small Animal Clinics of Madras Veterinary College, Chennai. Ruminant ticks namely *R. microplus, R.haemaphysaloides, H. bispinosa* and *H. marginatum* were collected from the cases presented in the Large Animal Clinics of Madras Veterinary College and Livestock Research Station, Kattupakkam. The above species of ticks were used in the current study. Engorged female ixodid ticks were collected from dogs and ruminants. They were placed in dry vials covered with porous cloth. These ticks were maintained in the laboratory for oviposition. Hatched larvae of all species were maintained at a RH of 93 per cent using saturated solution of potassium chloride. The unfed larval stages were maintained alive by keeping them at 4 ^{0}C in a BOD which ensured dormancy of larvae. Larval ticks with intact first pair of legs were only selected for use in the bioassays since the Haller's organ is located on the dorsal surface of the tarsus of the forelegs [9].

2.2. Filter paper

Whatman® qualitative filter paper grade 3 having a diameter of 11 cm (Whatman International Ltd., Maidstone, England) was used to impregnate volatile fatty acids [10]. Filter paper discs of size 2x2 cms were used in petridish bioassay. The discs were handled with a gloved hand. Sterile forceps were used to handle the filter paper discs. This ensured that the filter paper discs did not come into contact with human skin lipids which were found attractive to ticks [11].

2.3. Volatile fatty acids

Behavioural responses were recorded to each of the ruminant tick species to simulate ruminant fluid odour that contains butyric and propionic acids. The efficacy of butyric acid and propionic acid (Sigma Alderich, Germany) were tested separately and in combination also. For individual trials 2 µl of butyric acid and propionic acid were used. For combination trials 1 µl each of butyric acid and propionic acid were used. Volatile fatty acids were applied on to the filter paper discs and were allowed to evaporate. One hundred larvae of the five tick species were evaluated using petri dish bioassay. Untreated filter paper discs served as control.

2.4. Petridish Bioassay

A method followed by Yoder and Stevens (2000) was adopted in this study with modifications. Glass petri dishes of 9 cm diameter were used for the assay. Filter paper discs of size 2×2 cms were pasted at one quadrant of the petri dish. Larvae were placed 10 at a time in the bioassay arena, opposite to the filter paper discs. Tests were replicated until N=100 for the larvae. Petri dish was covered with another petri dish of same diameter and sealed with laboratory grade parafilm in order to prevent the escape of ticks as well as to avoid responses by the tick to carbon dioxide emitted by the investigator which could lead to misinterpretation of results. Care was taken while handling the ticks in order to avoid damage of the first pair of legs. Controls were also maintained in separate petri dishes. All tests were conducted at room temperature (27-33 ^{0}C). Unconditioned ticks (ticks which were unexposed to the semi chemicals), fresh chemicals, filter paper discs, petri dishes were used for each test. Continuous monitoring for 1 hour was done and the number of ticks that were attracted at 1 hour onto the disc or in the same quadrant of source was counted.

3. Statistical analysis

Statistical analysis of the data obtained was done with chi- square test.

4. Results and Discussion

Attraction to rumen metabolites represents a fundamental resource tracking adaptation by hard ticks in large mammals [12]. The chemical eructed from the rumen to the exterior is significant in mediating hard tick recruitment to suitable host. Ixodid ticks quest to locate and identify the host so as to enable parasite engagement through direct contact with the passing animal. Host finding is achieved by sequence of behavioural phases of which response to volatiles emanating from the host is an important one [5]. Reports indicate that ixodid ticks are activated by all mammalian odours but some ticks such as larval *R. microplus* were more activated by bovine odours [9]. In the present study Larvae when released into the petri dish bio arena containing the volatile fatty acid showed immediate questing response. The larvae were attracted either to the filter paper discs or in the same quadrant of the source. Those larvae which reached the impregnated filter paper assumed 'feeding posture' with lowered mouth parts touching the filter paper discs and indulged in continuous probing behaviour. Such reactions were evident within 10 minutes of release of the larvae into the bioassay arena. The response of the larvae of *R. microplus, R.haemaphysaloides, H. bispinosa* and *H. marginatum* is as shown on Tables 1. There was no characteristic behavioural response among the larval stages to the untreated control filter paper discs.

Table 1: Effect of the volatile fatty acids on ixodid tick larvae

Volatile fatty acid used → Tick species used (N=100 for each trial) ↓	**Butyric acid - 2µl**					**Propionic acid - 2µl**					**Butyric + Propionic acid - 1µl each**				
	No. of larvae attracted to the kairomone			No. of larvae not attracted to the kairomone		No. of larvae attracted to the kairomone			No. of larvae not attracted to the kairomone		No. of larvae attracted to the kairomone			No. of larvae not attracted to the kairomone	
	On disc	same quadrant	Total	With Orientation	Without orientation	On disc	same quadrant	Total	With Orientation	Without orientation	On disc	same quadrant	Total	With Orientation	Without orientation
R.microplus	5 (13.52)	32 (86.48)	37 (37)	20 (20)	43 (43)	3 (10.71)	25 (89.29)	28 (28)	10 (10)	62 (62)	7 (16.28)	36 (83.72)	43 (43)	15 (15)	42 (42)
R.haemaphysaloides	4 (9.3)	39 (90.7)	43 (43)	21 (21)	36 (36)	7 (17.5)	33 (82.5)	40 (40)	19 (19)	41 (41)	11 (31.43)	24 (68.57)	35 (35)	24 (24)	41 (41)
H.bispinosa	8 (25)	24 (75)	32 (32)	18 (18)	50 (50)	6 (15.79)	32 (84.21)	38 (38)	19 (19)	43 (43)	2 (9.09)	20 (90.91)	22 (22)	8 (8)	70 (70)
H.marginatum	3 (6.97)	40 (93.03)	43 (43)	18 (18)	39 (39)	3 (6.97)	40 (93.03)	43 (43)	16 (16)	41 (41)	1 (2.17)	45 (97.83)	46 (46)	17 (17)	37 (37)
Chi-square value and significance			3.57^{NS}					5.42^{NS}					14.89^{**}		

NS-Non significant ($p \geq 0.05$), ** Highly Significant ($p \leq 0.01$), Figures in parenthesis indicate percentage

Comparison of the level of response of all five ixodid tick larvae to volatile fatty acids like butyric acid, propionic acid and a combination was done (Fig.1). The study revealed that *H. marginatum* and *R.haemaphysaloides* showed maximum attraction to butyric acid (43 %) where as *H. bispinosa* was least attracted (32 %). With propionic acid *H. marginatum* revealed highest attraction (43 %) followed by *R. haemaphysaloides* (40 %). The least attraction towards propionic acid was exhibited by *R. microplus* larval stage (28 %). Combination of propionic acid and butyric acid resulted in maximum attraction of 46 per cent among *H. marginatum* followed by *R. microplus* larvae (43 %). The least attraction to the combination trial was exhibited by *H. bispinosa* larvae. *Rhipicephalus sanguineus* larvae did not exhibit any attraction or behavioural response towards the volatile fatty acids.

Amongst the ruminant ticks, *H. marginatum* larvae showed 43 % attraction to both butyric and propionic acid. When the larvae that showed orientation towards the source was considered the percentages of attraction was much higher for butyric and propionic acid. Combination of butyric acid and propionic acid also resulted in higher percentage of attraction. *H. marginatum* is a hunter tick with wide host spectrum and hence probably responded to the specific olfactory stimulation in a more specific manner than other ruminant ticks. Similarly *R.haemaphysaloides* also showed attraction to butyric acid and propionic acid comparable to that of *H. marginatum*.

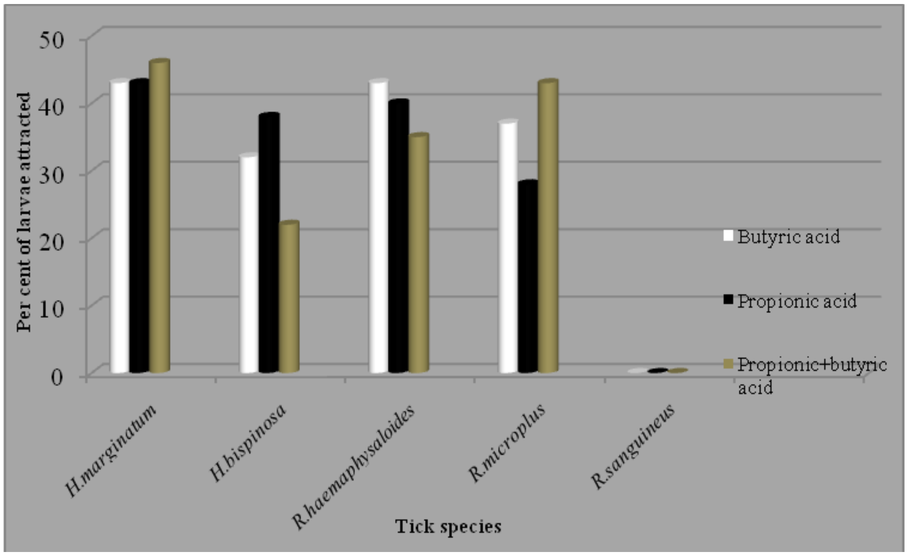

Fig 1: Comparison of the effect of volatile fatty acids on the larvae of ixodid ticks

Rhipicephalus microplus is a one host tick with narrow host range namely bovines. The tick is peculiar in that only the larvae performs host seeking behaviour. Hence it would appear to be advantageous for the highly host specific *R. microplus* to identify an approaching host before attachment. In the present study the response of *R. microplus* to butyric and propionic acid individually was moderate while a blend of butyric acid and propionic acid resulted in higher attraction. It has already been documented that cattle tick always prefer bovine odours [5]. It is assumed that a synergestic mixture of odours is required for higher percentages of attraction since this behavioural response is to specific olfactory stimulation. Since no work has been done on *H. bispinosa* which is predominantly a small ruminant tick it is assumed that propionic acid stimulating 57 % positive response from the tick larvae would be used to attract *H. bispinosa* larvae. The combination of butyric and propionic acid was ineffective in attracting substantial number of larvae. The dog tick *R. sanguineus* was also exposed to the ruminant volatile fatty acid (s) and as expected it did not respond to any of the volatile fatty acid or their combinations since these are specific to ruminants.

Fig 2: Petridish bioassay

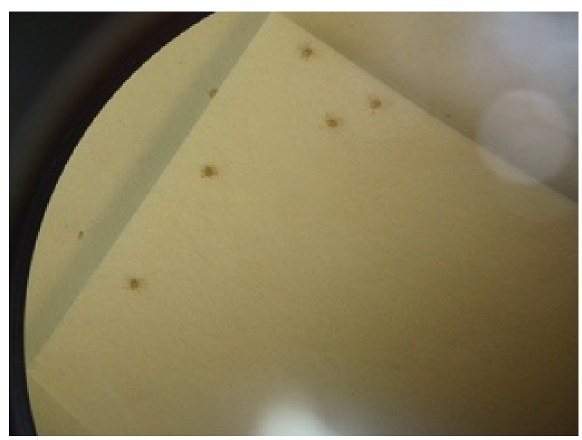

Fig 3: Attracted ixodid tick larvae on to the filter paper disc

Fig 4: *Hyalomma marginatum* larvae in feeding posture

5. Conclusion

In conclusion *H. marginatum* and *R.haemaphysaloides* showed maximum attraction to butyric acid (43 %) where as *H. bispinosa* was least attracted (32 %). With propionic acid *H. marginatum* revealed highest attraction (43 %) followed by *R. haemaphysaloides* (40 %). The least attraction towards propionic acid was exhibited by *R. microplus* larval stages (28 %). Combination of propionic acid and butyric acid resulted in maximum attraction of 46 % among *H. marginatum* followed by *R .microplus* larvae (43 %). The least attraction to the combination trial was exhibited by *H. bispinosa* larvae (22 %). *Rhipicephalus sanguineus* larvae did not exhibit any attraction or evince any behavioural response towards the volatile fatty acids. Statistical analysis revealed significant difference in the attraction response of all five ixodid tick species in response to both volatile fatty acids. Different volatile fatty acids emanating from ruminants can be tried for future studies. Similarly a synergestic mixture of volatile fatty acids simulating ruminant odour can be used to enhance the attraction efficacy.

6. References

1. Perry BD, Randolph TF, Mcdermott JJ, Sones KR, Thornton PK. Investing in animal health research to alleviate poverty. International Livestock Research Institute, Nairobi, Kenya. 2002.
2. Ghosh S, Azhahianambi P, Yadav MP. Upcoming and future strategies of tick control: a review. J. Vect. Borne Dis. 2007; 44: 79-89.
3. Jabbar A, Alp H, Aksin M, Seitzer U. Current status of ticks in Asia. Parasitol. Res. 2007; 101(2): S159-162.
4. Sonenshine DE. Biology of Ticks, Vol.1, Oxford University Press, New York, New York, 1991; pp 225-227.
5. Osterkamp J, Wahl U, Schmalfuss G and Haas W. Host odour recognition in two tick species is coded in a blend of vertebrate volatiles. J. Comp. Physiol. 1999; A185: 59-67.
6. Yoder JA, Stevens BW. Attraction of immature stages of the American dog tick (*Dermacentor variabilis*) to 2, 6–dichlorophenol., Exp.Appl.Acarol. 2000; 24: 159-164.
7. Hall DR, Beevor PS, Cork A, Nesbitt BF, Vale GA. 1-octen-3-ol: A Potent olfactory stimulant and attractant for tsetse isolated from cattle odours. Insect Sci.Appl. 1984; 5: 335-339.
8. Steullet P, Guerin PM. Identification of vertebrate volatiles stimulating olfactory receptors on tarsus I of the tick *Amblyomma variegatum fabricius* (Ixodidae) I. Receptors within the Haller's organ capsule. J. Comp.Physiol. 1994; A174: 27-38.
9. Sonenshine, DE. Tick Pheromones and their use in their control. Ann. Rev. Entomol. 2006; 51: 557-580.
10. Sonenshine DE. Chemical composition of some components of the arrestment pheromone of the black- legged tick, *Ixodes scapularis* (Acari: Ixodidae) and their use in tick control. J. Med. Entomol. 2003; 40(6): 849-859.
11. Yoder JA, Atwood AD, Stevens BN. Attraction to squalene by ticks (Acari: Ixodidae): First demonstration of a host derived attractant. Int. J. Acarol. 1998; 24: 159-164.
12. Donze G, McMahon C, Guerin PM. Rumen metabolites serve ticks to exploit large mammals. J.Exp. Biol. 2004; 207: 4283-4289.

Ranju RS, Latha BR, Leela V, Basith SA. Effect of ruminant kairomone volatile fatty acids on larval stages of five ixodid tick species. Journal of Zoology Studies. 2014; 1(3): 14-18

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