Effect of attractant sex pheromone on immature larval stages of ixodid tick species

R. S. Ranju · Bhaskaran Ravi Latha ·
V. Leela · S. Abdul Basith

Received: 19 April 2012 / Accepted: 21 June 2012 / Published online: 4 July 2012
© Indian Society for Parasitology 2012

Abstract Attractant sex pheromone (ASP) 2,6-dichloro phenol was used in the current study to evaluate the percentage attraction and the behavioural responses of the five ixodid tick species namely *Rhipicephalus sanguineus*, *Rhipicephalus microplus* (*Boophilus microplus*), *Haemaphysalis bispinosa*, *Rhipicephalus haemaphysaloides* and *Hyalomma marginatum* using petridish bioassay. Two concentrations of 2,6-DCP (0.1 M and 0.05 M) was used for the larval stages of all five ixodid tick species of which 0.1 M concentration was found to have maximum attraction. Trials with 0.1 M ASP revealed highest per cent of attraction in *R. sanguineus* larvae (71 %) followed by *H. bispinosa* (55 %) and *R. microplus* (55 %). With 0.1 M ASP *R. haemaphysaloides* and *H. marginatum* showed least attraction (39 % each). However the per cent of attraction of *R. haemaphysaloides* was higher (46 %) with 0.05 M ASP. Statistical analysis revealed a highly significant difference in per cent of attraction between the different tick larvae using 0.05 and 0.1 M ASP. The larvae also exhibited behavioural responses such as feeding, probing, resting and questing posture.

Keywords 2,6-DCP · Ixodid tick larvae · Behavioural response · Attractant sex pheromone

Introduction

Ticks have a key role in disease transmission and pose a major constraint in the development and improvement of livestock industry. Ticks surpass all other arthropods in the number and variety of pathogen they can transmit to domestic animals, and are ranked second next to mosquitoes as vectors of human diseases (Sonenshine 1993). 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. *Rhipicephalus sanguineus* transmits ehrlichiosis, babesiosis and hepatozoonosis while the ruminant ticks are the vectors for theileriosis, babesiosis and anaplasmosis. In India, the economic losses due to tick and tick-borne diseases are estimated to be US \$498.7 million per annum (Ghosh et al. 2007) with a global annual loss of \$109 billion (Jabbar et al. 2007). Currently the mainstay of tick control measure relies only 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 (Dipeolu and Ndungu 1991), apart from the worrisome selection of acaricide resistant ticks. An effective alternative tick control method used in other countries includes semiochemicals (Sonenshine 2003). Exploitation of this eco friendly method has not been attempted in India.

2,6-Dichlorophenol (2,6-DCP) has been proved to be the attractant sex pheromone (ASP) in metastriate ticks. This

R. S. Ranju · B. R. Latha · S. A. Basith
Department of Veterinary Parasitology, Madras Veterinary
College, Tamil Nadu Veterinary and Animal Sciences
University, Chennai 600007, Tamil Nadu, India

V. Leela
Department of Veterinary Physiology, Madras Veterinary
College, Tamil Nadu Veterinary and Animal Sciences
University, Chennai 600007, Tamil Nadu, India

R. S. Ranju (✉)
No. 11, Pary Street Extension, Choolaimedu,
Chennai 600094, Tamil Nadu, India
e-mail: drranjuvet@gmail.com

compound has been reported from six genera of ticks. Only fed or feeding male ticks can detect this compound (Sonenshine 2003, 2004, 2006). Unfed males can detect the pheromone but do not respond to it (Haggart and Davis 1981). It also acts as an attractant stimulus for immature ticks (Yoder and Stevens 2000). The observation of 2,6-DCP in various stages of ticks is consistent with the hypothesis of Yoder et al. (2002) linking chlorophenol production in ticks to water conservation. In an environment saturated with 2,6-DCP males were not able to detect females and did not mate (Ziv et al. 1981). Thus the control became possible by confounding males or mimicking females. Males were attracted to inanimate objects coated with 2,6-DCP. Sonenshine et al. (1985) observed that the mating frequencies in the dog ticks that received pheromone were reduced to 11.4 %. Use of this chemical prompted the expression of a novel type of feeding posture behaviour in adult ticks of *Dermacentor variabilis*. Mounting and ventral positioning were observed with lower concentration of 2,6-DCP. A complete behaviour sequence of orientation, location, mounting and ventral positioning were observed in *Anocentor nitens* ticks suggesting the role of 2,6-DCP as an attractant and mounting sex pheromone in these species (Borges et al. 2002). This paper explores the attraction and behavioural responses of 2,6-DCP on the five ixodid larval stages.

Materials and methods

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 dogs 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. The hatched out larvae of all species were maintained at a RH of 93 per cent using saturated solution of potassium chloride. The hungry larval stages were maintained alive by keeping them at 4 °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 (Sonenshine 2006).

Filter paper

Whatman® qualitative filter paper grade 3 having a diameter of 11 cm (Whatman International Ltd., Maidstone, England) was used to impregnate both the assembly pheromone and acaricide (Sonenshine 2003). Filter paper discs of size 2 × 2 cm were used in petridish bioassay. The discs were handled with a gloved hand. Sterile forceps were used to take 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 (Yoder et al. 1998).

Attractant sex pheromone (ASP)

Attractant sex pheromone 2,6-DCP was obtained from Sigma Alderich, Germany. Using HPLC grade reagent acetone (Sigma Alderich) 0.05 M, 0.1 M and 1 M solutions of ASP was prepared. A pilot trial using 100 larval stages of *R. microplus* was conducted to evaluate the effect of 0.05, 0.1 and 1 M solution of ASP. Based on the results 0.1 and 0.05 M solution of ASP was selected for further trials. Acetone (25 µl) served as control. Behavioural responses and the per cent of attraction of larvae of all the five species of ixodid ticks were observed using petridish bioassay. Hundred larvae of each ixodid tick species were used for individual assay.

Petridish bioassay

A method followed by Yoder and Stevens (2000) was adopted in this study with modifications. Glass petridishes of 9 cm diameter were used for the assay. Filter paper discs of size 2 × 2 cm were pasted at one quadrant of the petridish. 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. Petridish was covered with another petridish 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 maintained in separate petridishes. All tests were conducted at room temperature (27–33 °C). Naive ticks (ticks which were unexposed to the semiochemicals), fresh chemicals, filter paper discs, petridishes were used for each test. Number of larvae that were attracted onto the disc or in the same quadrant of source was counted. Observations were made up to 1 h.

Statistical analysis

Using χ^2 test statistical analysis of the data was done to compare the effect of ASP between the different tick species.

Results and discussion

2,6-DCP has been reported from 6 genera of ticks including 15 different species (Sonenshine 2006). Female ticks produce this pheromone soon after the nymphal moult from the foveal glands. Both male and female ticks are having foveal glands but female tick foveal glands are larger and contain more quantity of sex pheromone than males (Sonenshine 2004). The foveal glands in larvae and nymphs are not active and only less amounts of 2,6-DCP has been secreted by these stages.

Although sex pheromones by definition are compounds or mixtures of compounds secreted by individual of one sex to attract individuals of opposite sex, reports state that 2,6-DCP was attractive to unfed nymphs and unfed larvae in *D. variabilis* ticks (Yoder and Stevens 2000). Hence in the present study larval response to 2,6-DCP was evaluated. Attractant sex pheromone elicited an immediate behavioural response amongst the tick larvae. The Tick larvae exhibited immediate questing response. With 0.05 M ASP the larvae of all the 5 ixodid tick species showed an attraction ranging from 23 to 46 % whereas with 0.1 M ASP the per cent of attraction varied from 39 to 71. Among the five ixodid tick species except *R. haemaphysaloides* attraction of the larvae was more with 0.1 M solution of ASP. The highest attraction was observed with *R. sanguineus* larvae (71 %) followed by *H. bispinosa* (55 %) and *R. microplus* (55 %) when 0.1 M ASP was used. With 0.1 M ASP *R. haemaphysaloides* and *H. marginatum* showed least attraction (39 % each). However, the per cent of attraction of *R. haemaphysaloides* was higher (46 %) with 0.05 M ASP (Table 1). Statistical analysis revealed a significant difference in per cent of attraction between the different ticks using 0.05 and 0.1 M ASP.

All the larvae in 2,6-DCP treated petridishes showed behavioural postures such as feeding postures, probing postures, resting postures and questing postures which clearly indicates the positive response of the larval stages to the sex pheromones. In feeding posture the tick will be with a lowered mouthparts. Probing posture was exhibited by a repeated to and fro movement of the mouthparts attempting for inserting the mouthparts into the pheromone treated filter paper disc. In resting posture the tick will curl its front pair of legs under its body and it will be akinetic.

Table 1 Comparison of the effect of attractant sex pheromone (ASP) on the larvae of ixodid tick species

Tick species	No. of larvae attracted near the source within 1 h	
	With 0.05 M 2,6-DCP	With 0.10 M 2,6-DCP
<i>Rhipicephalus sanguineus</i>	43	71
<i>Rhipicephalus microplus</i>	36	55
<i>Hyalomma marginatum</i>	23	39
<i>Haemaphysalis bispinosa</i>	36	55
<i>Rhipicephalus haemaphysaloides</i>	46	39
χ^2 value and significance	13.54**	28.17**

** Highly significant ($p \leq 0.01$)

The sideways movement of the front pair of legs in order to perceive the smell of the surroundings were formed as questing posture. This posture was used to locate the odour source. Larval ticks showed neither attraction nor any behavioural responses to control filter paper discs.

Feeding stimulates the foveal glands to secrete 2,6-DCP (Sonenshine 1991). The feeding posture behaviour documented implies that 2,6-DCP is not exclusively a sex pheromone and that it might have an additional role as an attachment stimulus (Norval et al. 1991). But restricted emission of 2,6-DCP in larval and nymphal stages of the ticks makes them not to function as a sex pheromone. However Sonenshine (1991) opines that the reaction of the larvae to 2,6-DCP was not specific to it but that the larvae were responding to the general category of substituted phenols. Once the ticks reaches the filter paper discs they assume probing postures. The motionless resting postures results in cluster formation of the tick larvae around the pheromone treated filter paper discs.

In conclusion of the two concentrations of 2,6-DCP used for the larval stages of all five ixodid tick species, 0.1 M concentration was found to have maximum attraction. Trials with 0.1 M ASP showed highest per cent of attraction in *R. sanguineus* larvae (71 %) followed by *H. bispinosa* (55 %) and *R. microplus* (55 %). With 0.1 M ASP *R. haemaphysaloides* and *H. marginatum* showed least attraction (39 % each). However the per cent of attraction of *R. haemaphysaloides* was higher (46 %) with 0.05 M ASP. Statistical analysis revealed a highly significant difference in per cent of attraction between the different tick larvae using 0.05 and 0.1 M ASP. So ASP can be effectively utilised to attract the questing larval and nymphal stages of ixodid ticks from the field. Future investigations in this area using 2,6-DCP in combination with an effective acaricide will help to effectively control the off host stages of ticks.

References

- Borges LMF, Eiras AE, Ferri PH, Lobo ACC (2002) The role of 2,6-dichlorophenol as sex pheromone of the tropical horse tick *Anocentor nitens* (Acari: Ixodidae). *Exp Appl Acarol* 27:223–230
- Dipeolu OO, Ndungu JN (1991) Acaricidal activity of “kupetaba” a ground mixture of natural products against *Rhipicephalus appendiculatus*. *Vet Parasitol* 38:327–338
- Ghosh S, Azhahianambi P, Yadav MP (2007) Upcoming and future strategies of tick control: a review. *J Vector Borne Dis* 44:79–89
- Haggart DA, Davis EE (1981) Neurons sensitive to 2,6-dichlorophenol on the tarsi of the tick, *Amblyomma americanum* (Acari: Ixodidae). *J Med Entomol* 18:187–193
- Jabbar A, Alp H, Aksin M, Seitzer U (2007) Current status of ticks in Asia. *Parasitol Res* 101(2):S159–S162
- Norval RAI, Peter T, Yunker CE, Sonenshine DE, Burrige MJ (1991) Response of the ticks *Amblyomma hebraeum* and *A. variegatum* to known or potential components of the aggregation–attachment pheromone. I. Long range attraction. *Exp Appl Acarol* 13:11–18
- Sonenshine DE (1991) *Biology of ticks*, vol 1. Oxford University Press, New York, pp 225–227
- Sonenshine DE (1993) *Biology of ticks*, vol 2. Oxford University Press, New York, pp 447–456
- Sonenshine DE (2003) 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* 40(6):849–859
- Sonenshine DE (2004) Pheromones and other semiochemicals of tick and their use in tick control. *Parasitology* 129:S405–S425
- Sonenshine DE (2006) Tick pheromones and their use in their control. *Annu Rev Entomol* 51:557–580
- Sonenshine DE, Taylor D, Corrigan G (1985) Studies to evaluate the effectiveness of sex pheromone-impregnated formulations for control of populations of the American dog tick, *Dermacentor variabilis* (Say) (Acari: Ixodidae). *Exp Appl Acarol* 1:23–34
- Yoder JA, Stevens BW (2000) Attraction of immature stages of the American dog tick (*Dermacentor variabilis*) to 2,6-dichlorophenol. *Exp Appl Acarol* 24:159–164
- Yoder JA, Atwood AD, Stevens BN (1998) Attraction to squalene by ticks (Acari: Ixodidae): first demonstration of a host derived attractant. *Int J Acarol* 24:159–164
- Yoder JA, Hanson PE, Pizzuli JL, Sanders CI, Domingus JL (2002) Sex Pheromone production and its relationship to water conservation: studies on a trichlorophenol in the American dog tick, *Dermacentor variabilis* (Acari: Ixodidae). *Int J Acarol* 28:379–385
- Ziv M, Sonenshine DE, Silverstein RM, West JR, Gingher KH (1981) Use of Sex Pheromone, 2,6-dichlorophenol, to disrupt mating by American dog tick, *Dermacentor variabilis* (Say). *J Chem Ecol* 7:829–840