Field trials to attract questing stages of brown dog tick, *Rhipicephalus sanguineus* using tick pheromone–acaricide complex

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Abstract Evaluations were made to assess the efficacy of assembly pheromone, attractant sex pheromone and the kairomone carbon dioxide in attracting the questing larval nymphal and adult stages of the brown ear dog tick, *Rhipicephalus sanguineus*. Ticks attracted to one location were killed using the acaricide deltamethrin. Initially, simulated field trials were conducted to observe the percentage of attraction of ticks from different distances. Tick lure comprising of carbon dioxide as kairomone and 2,6-dichlorophenol in combination with assembly pheromone and deltamethrin on a thermocol platform was prepared and field trials were conducted in the kennels of Blue Cross Society of India, Velachery, Chennai. The tick lure attracted larval, nymphal and adult stages of *R. sanguineus* ticks in and around the kennels. Observations were made after 24 h of placing the tick lure. In simulated field out of the 50 ticks used for each distance trials, positive response was exhibited by a total number of 20 (40 %), 17 (34 %) and 14 (28 %) ticks from 0.5, 1 and 1.5 m, respectively. Field trials conducted revealed considerable attraction of larvae and nymph to the tick lure. Few adult ticks (2–5) were also lured to the pheromone impregnated filter paper disc.

Keywords Assembly pheromone · Attractant sex pheromone · Carbon dioxide · *Rhipicephalus sanguineus* · Tick control

Introduction

Ticks have attracted a great deal of scientific attention primarily because of their considerable medical and veterinary importance. Currently, the mainstay of tick control measure relies 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. These limitations point to an urgent need for novel tick control measure to reduce or to replace the use of acaricides, especially in the regions where extensive tick resistance has occurred. One of the new approaches proposed to improve tick control is the use of tick pheromones in combination with acaricides to attract and kill ticks. Pheromones include arrestment (assembly) pheromones (AP), attraction–aggregation–attachment pheromones and sex pheromones (Attachment sex pheromone, Mounting sex pheromone, Genital sex pheromone).

Waste products of nitrogen metabolism eliminated in tick excreta, comprise the assembly pheromone in Argasid ticks (Dusbabek et al. 1998). The response to this pheromone was in the form of clustering of ticks in caves, under ledges, cracks and crevices which enhanced the mating and host finding success (Sonenshine 1985). The main compound of the pheromone is the purine guanine, but xanthine, hypoxanthine and uric acid were also identified in the excreta of some ticks (Hamdy 1972, 1973; Otieno et al. 1985; Dusbabek et al. 1991). The compounds like

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hypoxanthine, xanthine, inosine, adenine and hematin from exudates or cast skins affected the behaviour of nymphs as well as adult males and females. 2,6-dichlorophenol (2,6-DCP) has been proved to be the attractant sex pheromone (ASP) in metastriate ticks. This compound has been reported from six genera of ticks (Sonenshine 2003, 2004, 2006). It also acts as an attractant stimulus for immature ticks (Yoder and Stevens 2000). Norval et al. (1989) stated that carbon dioxide initiated active and non-directional host finding behaviour in ticks. Carbon dioxide emitted by the host stimulates resting ticks in the environment which then emerge from the shelter (Hess and de Castro 1986; Steullet and Guerin 1992). Kairomone used in the current study was carbon dioxide. Price et al. (1994) proposed that carbon dioxide alerted the ticks to the host presence and pheromone attracted the ticks to particular hosts. In vitro evaluations in MVC, Chennai revealed 71, 70 and 76 % attraction of *Rhipicephalus sanguineus* ticks with 0.1 M ASP, AP–deltamethrin combination and carbon dioxide, respectively. This paper explores the on field efficacy of a combination of AP, ASP, Carbon dioxide and deltamethrin on the questing stages of the brown dog tick.

Materials and methods

Ticks

Ticks were located by visual appraisal and by running the hand across the body of the animal. Partially fed dog ticks, *R. sanguineus* for simulated field trials were collected from the animals presented in the Small Animal Clinics of Madras Veterinary College, Chennai.

Filter paper

Whatman® qualitative filter paper grade 3 having a diameter of 11 cm procured from Whatman International Ltd., Maidstone, England was used to impregnate Pheromones and acaricides (Sonenshine 2003). For both simulated field trials and field trials filter paper discs having a size of 4 × 4 cm were made used. Tick decoy was prepared using filter paper strips of size 8 × 2 cm. 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).

Acaricide

Deltamethrin (1.25 %), a commercially available preparation of synthetic pyrethroid (Butox®, Intas Pvt. Ltd., Gujarat) was used as an acaricide in the current study. For

simulated field trials and field trials acaricide solution was prepared by diluting 400 µl of deltamethrin in 2.5 ml of diethyl ether (Fischer Chemic Ltd., Chennai) and for tick decoys 800 µl of deltamethrin in 2.5 ml of diethyl ether was used. The filter paper discs to be impregnated were kept in an air tight container and the acaricide solution was poured onto it. The container was made airtight by sealing with laboratory grade parafilm (Pechiney plastic packaging, Chicago) in order to prevent the evaporation of the diluent, diethyl ether. The filter paper discs were kept in the acaricide solution for 24 h for impregnation. Impregnated filter paper discs were taken out with forceps and were used for the assays.

Attractant sex pheromone

Attractant sex pheromone 2,6-DCP was obtained from Sigma Aldrich, Germany. Using HPLC grade reagent acetone (Sigma Aldrich) 0.1 M solution of ASP was prepared. Mixed well and 25 µl of the solution containing 0.411 mg of 2,6-DCP was used immediately for the trials.

Assembly pheromones

The synthetic analogues of the AP guanine, xanthine, adenine and hematin were procured from Sigma Aldrich, Germany. These were used in a ratio as indicated by Sonenshine (2004) with slight modifications. Guanine, xanthine, adenine and hematin were used in 25:1:1:1 ratio. A total of 105.6 mg of AP were used in this study. The mixture was diluted in 4 ml of 0.95 % normal saline, mixed well, stored at room temperature and was used 3 h after preparation.

Carbon dioxide

Dry ice was used as the source of carbon dioxide. For simulated field trials and field trials 500 and 200 g of dry were used respectively.

Simulated field trial

Thermocol platform of 15 × 15 × 1 cm³ dimension was prepared to place the pheromone–acaricide impregnated filter paper discs and a petridish containing carbon dioxide source. Acaricide impregnated filter paper discs were pinned firmly to the four quadrants of thermocol platform. The prepared solution of 2,6-DCP was applied in 25 µl quantities at the two opposite quadrants of the filter paper discs. AP in 200 µl quantities were applied in the remaining two quadrants. They were allowed to air dry. At the centre of the thermocol platform a 9 cm diameter petridish was placed with 500 g of dry ice. This was used as a lure in the

simulated field trial. Simulated field trials were conducted with 150 partially fed *R. sanguineus* adult male ticks (50 ticks for each distance) to evaluate the efficacy of ideal semiochemical–acaricide complex in attracting the tick from different distances. Ten ticks were released at a time for each trial and a total of 5 trials for each distance were performed. Ticks were released from 0.5, 1 and 1.5 m distances away from the lure. Control lures consisted of untreated filter paper discs and without carbon dioxide source. The number of ticks attracted within 30 min to the vicinity of the lure was noted.

Field evaluation of tick lure

Field trials were conducted in the kennels of Blue Cross Society of India, Velachery, Chennai. The lures were prepared based on the simulated field trial traps. Since 500 g dry ice used in simulated field trap showed a repellent tendency among ticks due to the cool air, only 200 g of dry ice was used as the source of carbon dioxide in field trials. These traps were placed at the corner of the kennels and in places of the kennels where there were cracks and crevices. Lures were placed in 10 empty kennels (one on each kennel). They were kept undisturbed overnight and the number of questing larvae, nymphs and adult ticks attracted to the lure were recorded after 24 h.

Tick decoy

The decoy trial was conducted on 10 tick infested dogs. To both ends of the impregnated filter paper strips 25 µl of 2,6-DCP was applied. At the centre of the filter paper strips 400 µl of assembly pheromone was applied and it was allowed to air dry. The decoy thus prepared was stapled onto a satin ribbon and it was tied around the neck of the dog. The collar was kept undisturbed overnight. The following day the decoy was observed for the presence of different stages of ticks.

Results and discussion

Assembly pheromones are well-known to produce arrestment/assembly responses both in argasid ticks and ixodid ticks. The cluster formation of these ticks is thought to enhance mating and host finding success (Sonenshine et al. 1985). Higher level of attraction was recorded when the AP were combined with an acaricide than when employed alone. The current trial made use of deltamethrin as acaricide of choice since deltamethrin is one of the presently used drug for tick control in India. Moreover the acaricides used should not be repellent to the ticks. 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 *Dermacentor variabilis* ticks (Yoder and Stevens 2000). In the current study carbon dioxide alerted the ticks to the host presence and pheromone attracted the ticks to particular hosts. Similar results were obtained by Price et al. (1994).

In simulated field trials positive response was exhibited by 20 (40 %), 17 (34 %) and 14 (28 %) number of ticks from 0.5, 1 and 1.5 m distances respectively. In this trial less attraction obtained could be due to failure in creation of artificial air flow towards direction of tick release and because the study was conducted in uncontrolled environmental conditions.

Field trials conducted in the kennels revealed considerable attraction of larvae and nymph to the tick lure. Few adult ticks (2–5 per filter paper strip) were also lured to the pheromone impregnated filter paper disc. The promising results obtained in this trial to attract and kill questing stages of the tick can be effectively utilised in off host control of ticks. The results thus indicate that there is an opportunity for a commercially viable product to be developed.

Out of the 10 dogs with tick decoy, three decoys revealed the presence of few dead adult ticks (3–4 ticks per decoy) after 24 h. However, the filter papers serving as decoy collars were found damaged in some cases. The disadvantage faced in this study can be overcome by impregnating the pheromone in a slow release device such as a plastic collar. Hamilton et al. (1989) had developed a device to attract mate seeking males to bead shaped plastic spherules using ASP and MSP. The attracted ticks were killed by using small quantities of toxicant in the plastic spherules. Micro capsules, plastic decoys, or a trap using rubber septum, hollow fibres, capillary filaments, poly ethylene or gelatine capsules or multi layer tags made of natural or synthetic polymer resins are other devices which can be used to serve as the female mimics or the decoys. Use of such devices is bound to improve the efficacy of the tick decoy for on host control of *R. sanguineus*.

In conclusion, tick lure and decoy using carbon dioxide, 2,6-DCP and AP were found to be effective both in off and on host control of ticks. Considerable attraction can be obtained by optimising the concentration of pheromone–acaricide complex. Further studies in this field will provide an effective eco friendly alternative tick control method.

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