

EFFECT OF ATTRACTION-AGGREGATION-ATTACHMENT PHEROMONE AND KAIROMONE, CARBON DIOXIDE ON BROWN DOG TICK, *RHIPICEPHALUS SANGUINEUS*

R.S. Ranju*, Bhaskaran Ravi Latha¹, V. Leela²

Department of Veterinary Parasitology, Madras Veterinary College,
Tamil Nadu Veterinary and Animal Sciences University, Chennai, Tamil Nadu, India – 600 007

ABSTRACT

In vitro evaluations were made to observe the efficacy of Attraction-Aggregation-Attachment pheromone (AAAP) and the kairomone carbon dioxide on partially fed adult ticks (Both male and female ticks) of *Rhipicephalus sanguineus* using olfactometer bioassay. Bioassays were conducted with 50 adult ticks each using three treatments namely AAAP in combination with carbon dioxide, carbon dioxide alone and AAAP alone. Dichloromethane (DCM), diluents of AAAP served as control. Experimental trials using AAAP along with carbon dioxide resulted in 38 per cent of *R. sanguineus* ticks showing attraction towards the pheromone source. AAAP alone attracted only 30 per cent of ticks while Carbon dioxide alone elicited an attraction of 76 per cent. The effect of AAAP on partially fed adult ticks of *R. sanguineus* was found to be not substantial. However carbon dioxide showed a marked attraction among the partially fed adult male ticks of *R. sanguineus*

Key Words - Attraction-Aggregation-Attachment pheromone (AAAP), carbon dioxide, *Rhipicephalus sanguineus*, olfactometer, (O)- Nitrophenol

INTRODUCTION

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). For many years, pyrethroids and organophosphates formulated as dusts, dips or collars were used on dogs to control ticks. However the use of acaricides is often accompanied by serious drawbacks such as

¹ Department of Veterinary Parasitology, Madras Veterinary College, Tamil Nadu Veterinary and Animal Sciences University, Chennai, Tamil Nadu, India – 600007

² Department of Veterinary Physiology, Madras Veterinary College, Tamil Nadu Veterinary and Animal Sciences University, Chennai, Tamil Nadu, India – 600007

*Corresponding author: R.S. Ranju

Present address / Address for correspondence: No. 9, Pasumarthy Street, Kodambakkam, Chennai, Tamil Nadu, India.
Permanent address: R.S. Nivas, Pralayagiri, Thokkadu P.O., Varkala, Thiruvananthapuram, Kerala, India – 695143.

E mail Id: drranjuvet@gmail.com, Phone: 09444220741

Part of M.V.Sc. thesis published

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.

Although work on control of tick by vaccination and ethno veterinary control has been attempted in India, use of tick pheromones and kairomones in tick control has not been exploited. Generation of data on these compounds in control of ticks thus becomes relevant to the Indian subcontinent. One of the tick pheromones used effectively against ticks in countries other than India includes Attraction-Aggregation-Attachment pheromone (AAAP). The Attraction-Aggregation-Attachment pheromones attract hungry ticks to a tick infested host and induce them to cluster together at a single location probing and attaching to the host skin. This group includes a mixture of organic volatiles secreted from the dermal glands of feeding males which appear to attract both male and female ticks. Schoni *et al.* (1984) first elucidated the chemical composition of AAAP and showed that it consists of a mixture of three organic volatiles namely o-nitrophenol, methyl salicylate and nonanoic acid in a ratio of 2:1:8 respectively. This pheromone has been reported from the genus *Amblyomma* (Obenchain 1984; Sonenshine 1993). No reports are available on its effect on the brown dog tick, *Rhipicephalus sanguineus*. This paper explores the effect of tick pheromone AAAP and kairomone carbon

dioxide on partially fed adult ticks of *R. sanguineus*.

MATERIALS AND METHODS

Ticks - Ticks were located by visual appraisal and by running the hand across the body of the animal. Dog ticks were collected from the animals presented in the Small Animal Clinics of Madras Veterinary College, Chennai. Speciation of collected ticks was performed with key identification of Walker *et al.* (2000). Partially fed male and female ticks were collected from the body of dogs. Ticks with intact first pair of legs were only selected and were used immediately after collection for the bioassays.

Filter Paper - Whatman® qualitative filter paper grade 3 having a diameter of 11 cm (Whatman International Ltd., Maidstone, England) was used to impregnate AAAP (Sonenshine, 2003). Filter paper discs of size 2x2 cms were used in olfactometer bioassays. 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).

Olfactometer Bioassay - A two choice T-tube olfactometer bioassay was used to assess the tick attraction to the test material or to the control (Figure 1). The olfactometer was designed based on Nchu *et al.* (2009) with minor modifications. It consisted of two square arms of 1x1 x10 cms and a stem of 1x1x5 cms size. The stem was connected tightly to the arms. The extreme ends of each of the arms and stem were connected to a square chamber of size 3x3x3 cms.

Ticks were released at the chamber marked as 'R'. Test and control filter paper discs were placed in each square chamber of the olfactometer namely marked T and C. Controlled air flow was maintained through each odour sources using an air pump with low, medium and high air current settings. For the current study, airflow was maintained at medium level, which was found to be convenient for the ticks. The flow rate at medium level was 5 ml/sec. Purification of the air entering in test and control chambers was ensured by using sterile millipore filters. The air with smell of the pheromone and controls were sucked out using a vacuum pump connected at the release point of the ticks (R). Humidity was maintained in the 'T' chamber (Test Source Chamber) using moistened cotton pads which were kept beneath the test and control odour source. This helped to maintain the relative humidity of the chamber at 70 ± 5 per cent level inside the olfactometer. Ticks were released one at a time into the 'R' chamber. Each tick was allowed a maximum of 5 minutes to respond to the pheromone/kairomones and at the junction of the T-tube ticks choose between the control and treatment arms. Ticks failing to respond after 5 minutes were removed. For each test dose 10 ticks were assayed. Trials were replicated till N=50 for the adult ticks. The position of treatment and control chambers were changed after each trial, by alternating the placement of control and test filter papers. The bioassay was conducted at a temperature of $30 \pm 2^\circ\text{C}$ and a relative humidity of 70 ± 5 per cent.

In the current study olfactometer bioassay was performed for assessing

the efficacy of Attraction – Aggregation – Attachment - Pheromone (AAAP). Dichloromethane (DCM), the diluent of AAAP served as control. The olfactometer was rinsed with DCM and was air dried at room temperature after each trial. The instrument was placed horizontally during the experiment to mimic the natural questing behaviour of ixodid ticks.

Attraction–Aggregation–Attachment Pheromone (AAAP) - Synthetic analogues of AAAP namely (*O*)-nitrophenol (Sigma Alderich, Germany), methyl salicylate (Sipali (1) Pharma, Chennai) and nonanoic acid (Sigma Alderich, Germany) in 2:1:8 ratio were prepared and used for the bioassays. Attraction-Aggregation-Attachment-Pheromone in a concentration of 0.022 mg was made use in the current study in a ratio of 2:1:8 (Nchu *et al.*, 2009).

Carbon dioxide - The pheromone was used in combination with 5 per cent carbon dioxide in the current study as per Nchu *et al.* (2009). Carbon dioxide cylinder was connected to the test arm (T) of the olfactometer (Fig. 1). Trials were conducted in the presence of carbon dioxide alone, carbon dioxide–AAAP and AAAP alone. Olfactometer bioassay was conducted with 150 partially fed adult male and female ticks of *R. sanguineus*. Fifty ticks each were used for AAAP alone trials, for AAAP–carbon dioxide trials and for carbon dioxide alone trial.

Statistical analysis - Statistical analysis of the data obtained was done with *chi-square test* (Finney, 1952).

RESULTS

In AAAP along with carbon dioxide trial 38 per cent of *R. sanguineus* ticks showed attraction towards the pheromone source. However AAAP alone attracted only 30 per cent of ticks. Carbon dioxide alone elicited an attraction of 76 per cent of the *R. sanguineus* ticks (Table 1). Statistical analysis revealed that there is a highly significant difference between the three treatments. The effect of AAAP on partially fed adult ticks of *R. sanguineus* was not substantial. However carbon dioxide acts as an effective kairomone in attracting the partially fed adults of *R. sanguineus*.

DISCUSSION

The effect of AAAP was evaluated only on the adult ticks of *R. sanguineus*. Attraction-Aggregation-Attachment-Pheromone is solely secreted by feeding male which attracted both male and female ticks. The female ticks appear to require this pheromone without which they will not attach and feed (Sonenshine 2006). The pheromone has been recorded only in certain species of genus *Amblyomma* (Sonenshine 1985; Obenchain 1984). In the current study, the response obtained to the AAAP alone was only 30 per cent whereas it was enhanced to 38 per cent when combined with carbon dioxide. However carbon dioxide alone resulted in 76 per cent attraction clearly indicating that in *R. sanguineus* AAAP does not play a prominent role while presence of carbon dioxide enhances the speed and proportion with which the ticks respond. Similar to these findings, Barre *et al.* (1997) also found that carbon dioxide itself is a very effective attractant,

producing an attraction of 43-60per cent among *A. variegatum* ticks. Although (O) -nitrophenol is supposed to stimulate searching and aggregation behaviour while methyl salicylate and nonanoic acid are believed to stimulate attachment, these responses were not evident in the dog tick during the current study. Differences in the components of AAAP may vary between ticks and that could be the reason for the unresponsive behaviour of *R.sanguineus* to this pheromone.

In conclusion, carbon dioxide was effective kairomone in attracting the adult ticks of *R. sanguineus*. However the effect of AAAP on partially fed adult ticks of *R. sanguineus* was not substantial. Further investigations using different concentrations of AAAP will reveal the role of this pheromone on the adults of dog ticks.

REFERENCES

- Barre, N., G.I. Garris and O.Lorvelec, 1997. Field sampling of the tick *Amblyomma variegatum* (Acari:Ixodidae) on pastures in Guadeloupe; attraction of carbon dioxide and/or tick pheromones and conditions of use. *Exp.Appl. Acarol.* 21: 95-108.
- Dipeolu, O.O. and J.N. Ndungu, 1991. Acaricidal activity of "kupetaba" a ground mixture of natural products against *Rhipicephalus appendiculatus*. *Vet. Parasitol.* 38: 327-338.
- Finney, D.J., 1952. Probit analysis – a statistical treatment of the sigmoid response curve. Cambridge University Press, Cambridge.
- Nchu, F., N.K. Maniania, A. Toure, A. Hassanali and J.N. Eloff,

2009. The use of a semiochemical bait to enhance exposure of *Amblyomma variegatum* (Acari: Ixodidae) to *Metarhizium anisopliae* (Ascomycota: Hypocreales). *Vet. Parasitol.* 160:279-284
- Obenchain, F.D., 1984. Behavioural interactions between the sexes and aspects of species specificity pheromone mediated aggregation and attachment in *Amblyomma*. In: *Acarology Vol.1* (ED Griffith, D.A. and Bowman, C.E.): 387-392, Ellis Horwood, Chichester.
- Schoni, R., E. Hoess, W. Blum and K. Ramstein, 1984. The aggregation-attachment pheromone of the tropical bont tick *Amblyomma variegatum Fabricius* (Acari:Ixodidae): isolation, identification and action of its components. *J. Insect Physiol.* 30: 613-618.
- Sonenshine, D.E., 1985. Pheromones and other semiochemicals of the Acari. *Ann. Rev.Entomol.* 30: 1-28.
- Sonenshine, D.E., 1993. *Biology of Ticks*, Vol.2, Oxford University Press, New York, New York, Pp: 447-456.
- Sonenshine, D.E., 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, D.E., 2006. Tick Pheromones and their use in their control. *Ann.Rev. Entomol.* 51: 557-580.
- Walker, J.B., J.E Keirans and I.G. Horak, 2000. Genus *Rhipicephalus* (Acari :Ixodidae) A guide to the brown ticks of the world. Cambridge University Press, Cambridge.
- Yoder, J.A., A.D. Atwood and B.N. Stevens, 1998. Attraction to squalene by ticks (Acari: Ixodidae) : First demonstration of a host derived attractant. *Int. J. Acarol.* 24: 159-164.

Table - 1 Effect of attraction – aggregation – attachment pheromone (AAAP) on the adults of *rhipicephalus sanguineus*

Pheromone/Kairomone used	No. of adult ticks attracted towards pheromone/kairomone	No. of adult ticks attracted towards the control
AAAP + Carbon dioxide	19 (38)	31 (62)
AAAP	15 (30)	35 (70)
Carbon dioxide	38 (76)	12 (24)
Chi-square value and significance	24.20**	

**Highly significant ($p \leq 0.01$), Figures in parenthesis indicate percentage

Fig. 1. Olfactometer with Carbon Dioxide

