RESEARCH ARTICLE
Open Access

Journal of Veterinary and Animal Sciences
ISSN (Print): 0971-0701, (Online): 2582-0605
https://doi.org/10.51966/jvas.2023.54.2.532-536

Adult immersion test for assessment of amitraz resistance in *Rhipicephalus sanguineus* ticks[#]

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Citation: Reena, S.N., Lakshmanan, B., Nair, S.N., Syamala, K., Radhika, R., Krishnanunni, P.K. and Chandana, S.D. 2023. Adult immersion test for assessment of amitraz resistance in *Rhipicephalus sanguineus* ticks. *J. Vet. Anim. Sci.* **54**(2):532-536
DOI: https://doi.org/10.51966/jvas.2023.54.2.532-536

Received: 15.11.2022 *Accepted: 09.01.2023* *Published: 30.06.2023*

Abstract

Ticks are obligate blood sucking ectoparasites recognised globally as vectors of various pathogens, next to mosquitoes. The present study is conducted on brown dog ticks collected from naturally infested dogs in and around Thrissur district. Adult immersion test was performed and the parameters such as engorged tick weight, egg mass weight as well as tick mortality were recorded to derive the reproductive indices, percentage inhibition of oviposition and percentage mortalities. Non-linear regression analysis of the dose response data yielded the LC_{50} values at 95% confidence intervals and, the slopes and regression coefficients of the different parameters were also derived. The Resistance factors (RF) were calculated to categorise resistance levels into I, II, III and IV. Resistance to amitraz was detected at level II in tick isolates collected from Choolissery and Mundur, at level III and level IV in isolates collected from Mannuthy and Paravattani, respectively. Two isolates collected from Mannuthy were found to be susceptible. One way ANOVA of resistance levels revealed that the resistance levels III and IV were significantly different from that of susceptible and resistance level II. This is the first report on detection of amitraz resistance levels in dog ticks from south India calling for proper implementation of tick control strategies to prevent spread of amitraz resistance.

Keywords: *Adult immersion test, brown dog ticks, resistance levels, Kerala*

[#]*Part of M. V. Sc. thesis submitted by first author to KVASU, Pookode*

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Ticks rely completely on the host blood for development of each life cycle stage which is the reason for their notoriety as carriers of several pathogens (Ghosh and Nagar, 2014). *Rhipicephalus sanguineus,* the brown dog tick or the kennel tick is a three-host tick and is distributed across the world. The ticks are responsible for spreading a number of pathogens causing canine disease conditions such as babesiosis, ehrlichiosis, hepatozoonosis and Q fever. Indiscriminate and frequent use of acaricides to control tick infestation has led to the development of acaricide resistance against majority of the drugs. A survey by FAO (2004) had revealed that tick population in India have become resistant to every acaricide available in the market. Inadequate managemental strategies, favourable climate and poor infrastructure facilities that offer favourable conditions for tick proliferation allowing ticks to survive throughout the year, have contributed to the development of acaricide resistance (Kumar *et al.,* 2020). Deltamethrin resistance to brown dog ticks have been established in Kerala (Anand *et al.,* 2021). Amitraz is a commonly used acaricide to control tick and mite infestations with its effects on mortality and reproduction. A meta-analysis of literature by Dzemo *et al.* (2022) had revealed that 254 out of 522 *Rhipicephalus (Boophilus) microplus* isolates collected and tested from ten different countries were resistance to amitraz. Only limited studies were conducted on evaluation of amitraz resistance in the dog ticks. Hence this study was carried out to explore the status of amitraz resistance in brown dog ticks.

Materials and methods

Collection of ticks

Engorged female ticks were collected from naturally infested dogs that were presented to the veterinary clinics, pet grooming centres, birth control centres, and from the dogs in kennels and animal shelters in and around Thrissur district. The ticks were collected in plastic vials covered with breathable cloth fabric to allow for ample air supply. Ticks collected from a group of animals were pooled and designated as an isolate, that were washed thoroughly and used for adult immersion test (AIT) within 24 hrs of collection as per Singh *et al*. (2014).

Acaricide

A total of 100 mg technical grade amitraz (Sigma-Aldrich, India) was diluted in 50 mL of 100% methanol for preparing the stock solution of 2000 ppm. Various dilutions of amitraz (10, 25 50, 100, 250, 500, 750, 1000 ppm) were prepared by diluting the stock solution in methanol for the experimental bioassay for each isolate.

Adult Immersion Test (AIT)

The engorged ticks were weighed and washed with distilled water to remove debris. Ticks in treatment groups were exposed to amitraz by immersing them into different working solutions for 2 min and the ticks in control group were immersed only in the diluent. Constant stirring of the ticks in the solution was carried out to ensure adequate exposure to the acaricide. After this, the ticks were dried with tissue papers and stored in glass vials covered with cloth fabric which were kept in the desiccator for 14 days to ensure favourable conditions for egg laying. Each concentration was replicated twice with 6-10 ticks per replication. Ticks that did not oviposit even after 14 days were considered dead. The parameters, *viz.* mortality, engorged tick weight and egg mass weight were recorded to calculate the resistance factors (RF), reproductive index (RI) and percentage inhibition of oviposition (%IO) using the formulae (Singh *et al.*, 2014)

Reproductive Index (RI) =
egg mass weight / engorged tick weight

Percentage inhibition of oviposition (%IO) =
[(RI control – RI treated)/RI control x 100]

Statistical analysis

The data analysis was carried out in the GraphPad Prism 5 software. Non-linear regression analysis of the log dose concentrations and mortality percentages was applied to calculate the LC_{50} at 95% confidence intervals (CI). The slopes and regression coefficients of mortality, RI and IO (%) were also derived. One way ANOVA of the obtained data on resistance levels and LC_{50} was carried

out to ascertain significance levels. The RFs were calculated as the quotient between LC_{50} of field tick isolate and LC_{50} of field susceptible strain of *R. sanguineus s. l.* obtained in the study. Depending on the RF, the resistance levels were characterised at level I (RF= 1.5-5), level II (RF= 5.1-25), level III (25.1-40), level IV (RF > 40) and susceptible (RF<1.4) (Sharma *et al.,* 2012).

Results and discussion

A total of eight isolates comprising of approximately 800 ticks were screened for amitraz resistance. Methanol was used as the diluent as amitraz was insoluble in water and organic solvent such as methanol facilitates the penetration and adsorption of acaricide across the exoskeleton of the tick (Sharma *et al.,* 2012). The LC_{50} (95% CI), R^2 (goodness of fit) values and slopes of mortality, along with the resistance factors and resistance levels were calculated for field isolates of *R. sanguineus s. l.* (Table 1). Non-linear regression graphs of mean mortality of ticks were plotted against the log concentrations of amitraz and the regression graphs of highly resistant ticks are provided in Fig.1 and Fig.2 (RL: III and IV). In the eight isolates screened, resistance to amitraz was detected in six isolates at levels II, III and IV whereas two isolates were susceptible.

In an isolate collected from Mannuthy from a dog reportedly unexposed to amitraz, 100 per cent mortality was observed at the lowest concentration of 10 ppm and hence regression analysis could not be performed. In another tick isolate collected from Mannuthy which was collected from a dog reportedly unexposed to amitraz, the LC_{50} calculated was 2.353 ppm which is the lowest LC_{50} recorded in the study, hence this isolate was used as the susceptible isolate to calculate the RFs. The LC_{50} value obtained in the study was nearer to the LC_{50} value of susceptible *R. (B). microplus* isolate derived by Dutta *et al.* (2017). The LC_{50} values of the remaining six isolates were in the range of 11.67-274.8 ppm which were in agreement with the LC_{50} ranges (27.5-77.4 ppm) of Dutta *et al.* (2017).

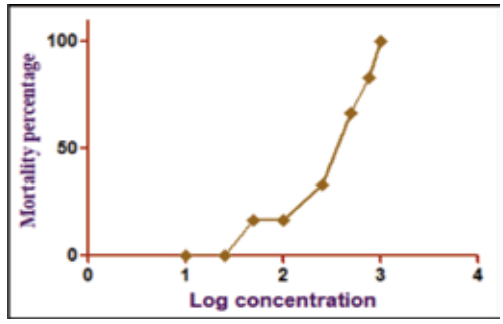

Fig.1. Regression plot of Paravattani isolate

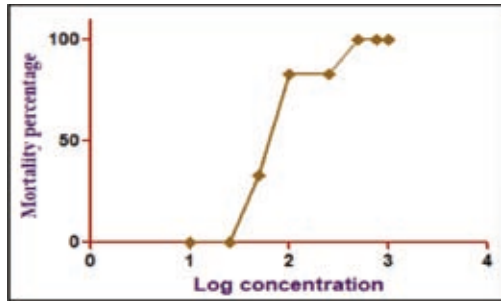

Fig.2. Regression plot of Mannuthy isolate

Table 1. Categorisation of resistance levels based on AIT

Locality of collection	LC_{50} (in ppm)	LC_{50} at 95% C. I.	R^2	Slope	Resistance factor (R.F)	Resistance level (R.L)
Choolissery	33.94	22.79 to 50.56	0.9274	35.7103	14.42	Level II
Choolissery	19.76	12.27 to 31.80	0.883	34.66898	8.39	Level II
Mundur	20.44	14.11 to 29.62	0.9736	35.69864	8.68	Level II
Mundur	11.67	4.699 to 29.01	0.8512	34.56759	5.38	Level II
Paravattani	274.8	156.8 to 481.5	0.9087	33.54259	116.7	Level IV
Mannuthy	68.29	30.21 to 154.3	0.8683	42.78728	29.02	Level III
Mannuthy	Not calculated since cent per cent mortality was observed at the lowest concentration					
Mannuthy	2.353	1.796 to 3.082	0.9236	27.38242	1	Susceptible

The highest LC_{50} observed was 274.8 ppm in an isolate collected from Paravattani that showed a resistance level IV. The LC_{50} value for Mannuthy isolate was 68.29 ppm which was categorised at resistance level III. Isolates collected from Choolissery and Mundur had LC_{50} values at a range of 11.67-33.94 that were categorised as level II resistant. Similar studies employing AIT in cattle ticks were conducted by Dutta *et al.* (2017) in Jammu and Kashmir reporting level I resistance, Singh *et al.* (2013) in Gujarat who reported level II resistance, Singh *et al.* (2014) in Punjab who reported level II resistance and Kumar *et al.* (2014) who reported resistance levels I, II and III from northern and eastern states of India.

Adult Immersion Test with Discriminating Dose (DD) was not conducted, as Jonsson *et al.* (2007) showed that DD concept is not effective for amitraz, by comparing both the classical AIT and AIT-DD in two series of experiments. One way ANOVA (Tukey's multiple comparison test) with the resistance levels and the LC_{50} values of the ticks from all the isolates revealed that no significant difference exists between susceptible Vs resistance level II. While the difference between susceptible and resistance levels III and IV were highly significant with a p-value of <0.05 (Table 2).

Effects of amitraz on egg laying were studied by comparing the egg masses of different isolates exposed to different concentrations of amitraz to that of the controls, which was achieved by calculating the slopes of RI and IO (%). The slopes obtained for RI were in the range of -0.228 to -0.1197 which were nearer to the slopes of RI obtained by Dutta *et al.* (2017) and Kumar *et al.* (2014). The negative slopes of RI in all the isolates indicated that reproduction (egg laying) was adversely affected by amitraz. Positive slopes were obtained with the IO (%) ranging between 23.22 to 37.58 which also suggested that as concentration of amitraz increases the egg laying capacity was inhibited implying that the efficiency of converting live weight into egg mass was affected among the surviving ticks which was in agreement with the range of slopes of IO (%) arrived by Dutta *et al.* (2017).

Studies employing the AIT to detect amitraz resistance were scarce, but a study employing AIT-DD in Karnataka by Rani *et al.* (2018) for evaluating the efficacy of three different acaricides in different species of ticks had shown that ticks were susceptible to amitraz. A study by Rodriguez-Vivas *et al*. (2016) in Mexico employing the larval immersion test to detect amitraz resistance revealed that low levels of resistance (RR= 1.68-4.58) were prevalent in dog ticks. Another study employing larval packet test (LPT) by Bandara *et al.* (2016) from Sri Lanka had also shown that low levels (RR= 1.52) of amitraz resistance is prevalent. There are no published records on amitraz resistance in brown dog ticks in India.

Conclusion

The present study detected the presence of amitraz resistance at levels II, III and IV in tick isolates collected from Thrissur district, Kerala. There are no available reports on amitraz resistance levels detection in brown dog ticks of India. The ticks attaining resistance levels III and IV is alarming, and if left undetected will lead to massive failure of the drug. The spread of amitraz resistance should be viewed seriously in the light of increasing ticks and tick-borne diseases and the indiscriminate use of this acaricide should be checked in the light of present study's findings of high levels of amitraz resistance. Alternative tick control strategies such as the employment of semiochemicals,

Table 2. Significance levels of adult immersion test

Tukey's Multiple Comparison Test	Mean Difference	Q value	Significance
Susceptible vs Level II	-19.13	4.785	NS
Susceptible vs Level III	-65.97	14.29	S
Susceptible vs Level IV	-272.5	59.03	S
Level II vs Level III	-46.84	11.72	S
Level II vs Level IV	-253.3	63.37	S
Level III vs Level IV	-206.5	44.74	S

NS – Non-significant S- Significant

phytoacaricides and vaccine development which are still in their infancy should be robustly studied. Further investigations on acaricidal resistance should be carried out involving different species of ticks to understand the true nature of acaricidal resistance in the state.

References

Anand, A., Lakshmanan, B., Kajal, T.A., Joseph, S., Aravindakshan, T.V. and Jose, J. 2021. Deltamethrin resistance in *Rhipicephalus sanguineus* and *Rhipicephalus (Boophilus) microplus* tick population in Kerala. *J. Vet. Ani. Sci.* **52:** 19-25.

Bandara, J., Dissanayake, D. M. A. P., de Silva, P. and Parakrama Karunaratne, S. H. P. 2016. First report on emerging resistance to Amitraz and Flumethrin in *Rhipicephalus sanguineus* (brown dog tick) at police kennels, Kandy, Sri Lanka: a case study. In: *Proceedings of Sri Lanka-Taiwan Joint Symposium on Advances in Entomological Research*. Postgraduate Institute of Science, University of Peradeniya, Sri Lanka. p. 20.

Dutta, S., Godara, R., Katoch, R., Yadav, A., Katoch, M. and Singh, N.K. 2017. Detection of amitraz and malathion resistance in field populations of *Rhipicephalus (Boophilus) microplus* (Acari: Ixodidae) in Jammu region of India. *Exp. Appl. Acarol.* **71**: 291-301.

Dzemo, W.D., Thekisoe, O. and Vudriko, P. 2022. Development of acaricide resistance in tick populations of cattle: A systematic review and meta-analysis. *Heliyon*. p.e08718.

FAO [Food and Agriculture Oganization]. 2004. Acaricide Resistance: Diagnosis, Management and Prevention. *Guidelines-Resistance Management and Integrated Parasite Control in Ruminants*. Animal Production and Health Division, Agriculture Department, Food and Agriculture Organization of the United Nations, Rome, pp. 25-77.

Ghosh, S. and Nagar, G. 2014. Problem of ticks and tick-borne diseases in India with special emphasis on progress in tick control research: a review. *J. Vector Borne Dis.* **51**: 259-270.

Jonsson, N. N., Miller, R. J. and Robertson, J. L. 2007. Critical evaluation of the modified-adult immersion test with discriminating dose bioassay for *Boophilus microplus* using American and Australian isolates. *Vet. Parasitol.* **146**: 307-315.

Kumar, S., Sharma, A.K., Ray, D.D. and Ghosh, S. 2014. Determination of discriminating dose and evaluation of amitraz resistance status in different field isolates of *Rhipicephalus (Boophilus) microplus* in India. *Exp. Appl. Acarol.* **63**: 413-422.

Kumar, S., Sharma, A.K. and Ghosh, S. 2020. Menace of acaricide resistance in cattle tick, *Rhipicephalus microplus* in India: Status and possible mitigation strategies. *Vet. Parasitol.* **278**: 108993.

Rani, R.S., ED'Souza, P., Byregowda, S.M., Veeregowda, B.M., Sengupta, P.P. Chandranaik, B.M. and Thimmareddy, P.M. 2018. *In vitro* acaricidal efficacy of deltamethrin, cypermethrin and amitraz against sheep ticks in Karnataka. *Exp. Parasitol*. **201**: 42-48.

Rodriguez -Vivas, R. I., Ojeda-chi, M. M., Trinidad-Martinez, I. and Bolio-Gonzalez, M. E. 2016. First report of amitraz and cypermethrin resistance in *Rhipicephalus sanguineus sensu lato* infesting dogs in Mexico. *Med. Vet. Entomol.* **32**: 72-77.

Sharma, A.K., Kumar, R., Kumar, S., Nagar, G., Singh, N.K., Rawat, S.S., Dhakad, M.L., Rawat, A. K. S., Ray, D. D. and Ghosh, S. 2012. Deltamethrin and cypermethrin resistance status of *Rhipicephalus (Boophilus) microplus* collected from six agro-climatic regions of India. *Vet. Parasitol*. **188**: 337-345.

Singh, N.K., Abhijit, N., Jyoti and Rath S.S. 2014. Detection of amitraz resistance in *Rhipicephalus microplus* from S.B.S Nagar, Punjab, India. *Sci. World J.* **2014**: 1- 4.

Singh, N.K., Gelot, I.S., Jyoti, Singh, V. and Rath, S.S. 2013. Detection of amitraz resistance in *Rhipicephalus microplus* from north Gujarat, India. *J. Parasit. Dis.* **39**: 49-52.

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