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Short Communication

Low level deltamethrin resistance in ticks from cattle of Kerala, a south Indian state

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ABSTRACT

The deltamethrin resistance status in *Rhipicephalus* (*Boophilus*) *annulatus* and *R.* (*B.*) *microplus* ticks collected from cattle of five organized farms of Kerala, south India was evaluated. Resistance was characterized using biological (larval packet test), biochemical (esterase enzyme activity assay) and molecular tools (PCR amplification and sequencing of deltamethrin resistance-associated genes). Characterization of field isolates revealed level I resistance in ticks collected from four out of five farms. Elevated level of α/β esterase activity was not recorded in isolates showing level I resistance. Previously reported point mutations in the carboxyl esterase (G1120A) and sodium channel (T2134A and C190A) genes were not observed in any of the field isolates. The present study showed a low level (level I) resistance is developed in the most economically important ticks infesting cattle of this state and it cautions the development of large scale resistance in future.

1. Introduction

Ticks are one of the important ectoparasites of domestic animals and ranked second to mosquitoes in their vector potentiality (Jonsson, 2006). Tick infestations lead to blood loss, skin and hide damage, tick toxicosis and tick worry in infested animals. According to FAO (1984), nearly 80 per cent of world's livestock populations are infested with ticks. Ticks and tick borne diseases (TTBDs) produced an estimated loss of US$ 13.9–18.7 billion globally (de Castro, 1997). In India, the annual control cost of TTBDs has been estimated as 498.7 million US$ (Minijauw and McLeod, 2003).

Kerala is a small state in the southwestern corner of India and is approximately 38,863 km^2 in size. Lying between north latitudes 8°18′ and 12°48′ and east longitudes 74°52′ and 72°22′, Kerala is well within the humid equatorial tropics. The seasons of Kerala can be broadly classified into hot and humid summer season from February to May, monsoon season from June to November and the fair season during December and January. The total annual rainfall in the state varies from 380 cm over the extreme northern districts to about 180 cm to the south. These conditions favor the propagation of the vector ticks.

Of the 106 tick species documented from India (Geevarghese et al., 1997), *R.* (*B.*) *annulatus*, *R.*(*B.*) *microplus*,

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R.(*B.*) *decoloratus*, *R. sanguineus*, *R. haemaphysaloides*, *R. turanicus*, *Haemaphysalis bispinosa*, *H. intermedia*, *H. aculeata*, *H. cuspidata*, *H. knobigera*, *H. turturis*, *H. spinigera*, *Hyalomma anatolicum*, *H. marginatum isaaci*, *H. hussaini*, *Amblyomma integrum*, *Nosomma monstrosum* and *N. keralensis* were reported from Kerala (Prakasan and Ramani, 2007) and *R.* (*B.*) *annulatus* is considered as one of the most economically important tick species of cattle (Ghosh et al., 2007).

The most widely used method of tick control in India and in other countries is the application of chemical acaricides, especially, synthetic pyrethroids (SP) *viz.*, deltamethrin, cypermethrin and flumethrin (Graf et al., 2004; Ghosh et al., 2007). Indiscriminate and widespread use of these chemicals led to the development of acaricidal resistance in ticks which has already been reported against almost all commercially available chemical compounds (Castro-Janer et al., 2009). In India, large scale acaricidal resistance against diazinon, deltamethrin and cypermethrin was reported from northern states (Kumar et al., 2011; Vatsya and Yadav, 2011; Sharma et al., 2012; Abdullah et al., 2012; Shyma et al., 2012). However, studies on acaricidal resistance status of economically important cattle ticks collected from southern India are very limited (Pradeep et al., 2012; Cattavarayane et al., 2013). The present communication is the first comprehensive study on deltamethrin resistance status in *R.* (*B.*) *annulatus* and *R.* (*B.*) *microplus* ticks infesting animals of selected organized farms of Kerala, southern India, using biological, biochemical and molecular biological tools.

2. Materials and methods

2.1. Collection of ticks

Targeted tick species were collected from five organized cattle farms (selected randomly) located at different regions *viz.*, Instructional Cattle Farm of the institute (PKD); Jersey Farm, Vithura, Thiruvananthapuram (TVM); Cattle Breeding Farm, Thumburmuzhi, Thrissur (THMB); Base Farm, Kolahalamedu, Idukki (KLMD) and Aiswarya Farm, Kannan Devan Hill Plantations Company Pvt. Ltd., Mattupetty, Idukki (MPTY) (Fig. 1) during active infestation period. Among these, four farms are owned and managed by the Government of Kerala and one by a private organization. All these farms were well maintained and farm activities like breeding, feeding and management are supervised by qualified veterinarian/s. The data on pattern of infestation and control methods adopted in these farms were collected. Adult engorged female ticks were kept in a Biochemical Oxygen Demand (BOD) incubator at $28 \pm 1\,^{\circ}C$ and $85 \pm 5\%$ relative humidity (RH) for egg laying and subsequent hatching of larvae.

2.2. Acaricide

Technical grade 100% pure deltamethrin (AccuStandard Inc., USA) was used to prepare 1000 ppm stock solution by diluting 10 mg of the compound in 10 ml of methanol. For experimental bioassay (larval packet test), different concentrations were prepared in distilled water.

2.3. Acaricide susceptible ticks

Two isolates, *R.* (*B.*) *annulatus* and *R.* (*B.*) *microplus* were collected from two places *viz.*, Mamalakkunnu, Meenangadi, Wayanad (MKN), northern Kerala and Livestock Research Station, Thiruvizhamkunnu, Palakkad (THVK), central Kerala respectively. Ticks from both the locations were susceptible to the recommended concentration of deltamethrin and were used as reference field susceptible stock for comparison of resistance.

2.4. Resistance detection

2.4.1. Determination of LC_{50} and LC_{95} values of reference susceptible tick isolate

For conducting larval packet test (LPT) (FAO, 1971) filter paper packets were prepared and impregnated with different concentrations (100–0.375 ppm) of deltamethrin. Treated packets containing live larvae were incubated for 24 h in BOD incubator at $28 \pm 1\,^{\circ}C$, $85 \pm 5\%$ RH. Three replicates of each concentration and two distilled water controls were used. After incubation, packets were checked for mortality of larvae. Mortality per cent was calculated. Dose–response data were analyzed by probit method (Finney, 1952) using Graph Pad Prism 4 software (GraphPad software Inc., USA). The 50% (LC_{50}) and 95% (LC_{95}) lethal concentrations of deltamethrin were determined by applying regression equation analysis to the probit-transformed data of larval mortality. All data were expressed as mean ± SEM. Groups were compared using one-way analysis of variance (ANOVA) for repeated measurements using SPSS software (IBM, USA). For *post hoc* analysis, Fishers' least square difference and Duncan tests were used. A value of $P < 0.05$ was considered as statistically significant.

2.4.2. Resistance characterization

Larvae of ticks collected from organized farms were tested at different concentrations of deltamethrin. Resistance factor (RF), the quotient between LC_{50} of field ticks and LC_{50} of field susceptible ticks (Castro-Janer et al., 2009) was determined. The resistance level (RL) in the field population of ticks was classified as susceptible ($RF \leq 1.4$), level I (RF = 1.5–5), level II (RF = 5.1–25), level III (RF = 25.1–40) and level IV (RF > 40) (Kumar et al., 2011).

2.4.3. Esterase enzyme assay

Esterase enzyme activity was assessed using α and β naphthyl acetate (Hemingway, 1998). Esterase activity in nmol of naphthol/minute/mg of protein was calculated. Esterase ratio was determined as the quotient between esterase activity of field isolates and esterase activity of reference susceptible isolates. The data were statistically analyzed with paired *t*-test using SPSS software (IBM, USA).

2.4.4. Molecular assay

Total RNA was isolated from 10 to 15 day old pooled larvae (approximately 30 mg) from 3 to 5 numbers of