Veterinary Parasitology 250 (2018) 30–34

Contents lists available at ScienceDirect

Veterinary Parasitology

journal homepage: www.elsevier.com/locate/vetpar

Research paper

Determination and validation of discriminating concentration of ivermectin against *Rhipicephalus microplus*

Abhijit Nandi[1], Sharath V. Sagar, Gajanan Chigure, Ashutosh Fular, Anil Kumar Sharma, Gaurav Nagar, Sachin Kumar, B.C. Saravanan, Srikant Ghosh*

Entomology Laboratory, Division of Parasitology, ICAR-Indian Veterinary Research Institute, Izatnagar, Bareilly, 243122 (UP), India

ARTICLE INFO

Keywords:
Discriminating concentration
Ivermectin
Resistance
Rhipicephalus microplus

ABSTRACT

Rhipicephalus microplus, the major cattle tick species of India is prevalent all over the country and causes huge economic loss directly or indirectly to the dairy industries. Chemical acaricides are playing an important role in managing tick infestations on livestock for many years and consequently, resistance to commonly used organophosphate (OP) and synthetic pyrethroid (SP) compounds has been reported. Subsequently, ivermectin (IVM) has been emerged as an alternative to manage OP and SP resistant ticks. However, with the increase of use during the last 5–8 years, there is a possibility of development of resistance and thus there is an urgent need to develop a robust resistance monitoring tool to safeguard the drug. Lethal concentrations for 50 and 95% mortality of treated ticks were determined to work out discriminating concentration (DC) in order to diagnose resistance in the field situation. The DC (2 x LC_{95}) was determined as 93.54 ppm using an established reference susceptible IVRI-1 line of *R. microplus* adopting adult immersion test. For validation of DC, the resistance status was checked in seven tick isolates of *R. microplus* collected from northern and eastern regions of India. The RR_{50} and RR_{95} values of the field isolates against ivermectin were determined and were in the range of 1.56–8.25 and 1.93–27.58, respectively. All the collected isolates were found to have higher lethal concentration and resistance ratio in comparison to reference susceptible IVRI-1 tick line (LC_{50} = 21.68, LC_{95} = 46.77 ppm, RR = 1.0). Amongst the field isolates, the isolate collected from Fatehgarh Sahib district (FTG) of Punjab state showed highest RR_{50} of 8.25 indicating high level of resistance to IVM. The generated DC will be used for IVM resistance characterization of ticks infesting cattle in different parts of the country.

1. Introduction

The discovery of Ivermectin (IVM) along with penicillin and aspirin has been considered as one of the major events in drug discovery research for its versatility, safety and beneficial impact on human and animal health. The IVM was discovered in 1975 and was the first commercially available broad-spectrum anti-parasiticide having ability to kill both internal and external parasites (Campbell, 1981; Putter et al., 1981; Crump and Ōmura, 2011). After extensive research, Merck and Co. introduced IVM and its analogues in the animal health market in 1981 and became world's largest selling animal health product, with a wide safety margin (Laing et al., 2017).

Ivermectin and other macrocyclic lactones are the most commonly used endo-ectocides in the livestock industry, particularly for the control of gastro-intestinal nematodes, lungworm and various ectoparasites including *Rhipicephalus microplus*, the major cattle tick of tropical and sub-tropical countries including India and cause significant economic loss directly or indirectly to the dairy industry (Ghosh et al., 2007). Different groups of chemical acaricide are in use for many years in tick infested countries for the management of ticks. However, due to indiscriminate and continuous use of the commonly available acaricides, resistance against different classes of acaricides *viz.* organophosphate (OP), synthetic pyrethroid (SP) and formamidine has been reported from different parts of the world (Li et al., 2003; Jonsson et al., 2007; Miller et al., 2002; Rosado-Aguilar et al., 2008; Raynal et al., 2013; Vudriko et al., 2016) and the reports are fast pouring in the data base. In India, after the establishment of discriminating concentration (DC) of commonly available acaricides, resistance data were enriched with a number of reports from different parts of the country (Kumar et al., 2011, 2014, 2015; Sharma et al., 2012; Singh et al., 2010, 2014; Shyma et al., 2015). It is observed that a number of tick isolates have developed resistance to multiple groups of acaricide (Ghosh et al., 2015).

* Corresponding author.
E-mail address: sghoshtick@gmail.com (S. Ghosh).
[1] Present address: Dept. of Veterinary Parasitology, West Bengal University of Animal and Fisheries Sciences, 37 K.B. Sarani, Belgachia, Kolkata, 700037, West Bengal, India.

https://doi.org/10.1016/j.vetpar.2017.12.009
Received 25 August 2017; Received in revised form 6 December 2017; Accepted 8 December 2017

Due to reduced efficacy of most of the commonly available acaricides, the use of injectable, oral and pour-on macrocyclic lactones (ivermectin, doramectin and moxidectin) has been increased and sporadic reports of IVM resistance is pouring in to the literature (Jaiswal et al., 2013; Singh et al., 2015; Gelot et al., 2016). To increase life span of the prime drug, it is necessary to have robust resistance monitoring system so that the process of development of resistance may be delayed.

In the established bioassay format for characterization of resistance, it is necessary to determine base line value of the toxicant against susceptible homogeneous reference populations. Employing homogeneous strains (Yeerongpilly and Mozo strains of *R. microplus)*, base line values and DC of macrocyclic lactones (MLs) against larvae and adults were determined in Australia (Sabatini et al., 2001) and in Brazil (Klafke et al., 2012). Similarly, for generation of base line value of the toxicant, a susceptible reference population (National registration no. NBAII/IVRI/BM/1/1998) is established in the entomology laboratory of the institute and a number of DCs against different group of acaricides were determined (Kumar et al., 2011, 2014, 2015).

Amongst the bioassays, the larval immersion test (LIT) (Shaw, 1966) was most popular for detecting IVM resistance (Perez-Cogollo et al., 2010; Castro-Janer et al., 2009) due to easy availability of good number of larvae for conducting the experiment. The problem mentioned by Jonsson et al. (2007) in conducting adult immersion test (AIT) using field isolates is partially true but we observed that larval based assay may not be the true alternative for AIT. To develop a suitable bioassay effective against most damaging stage of the parasite, the present study was conducted to determine and validate the DC of IVM using adults as starting material.

In AIT, freshly dropped fully engorged females are immersed in technical or commercial grade acaricides and the percentage mortality, rate of oviposition and egg masses were compared between treated and untreated groups. Although Jonsson et al. (2007) reported that the limiting factor for the AIT is the non-availability of sufficient number of adult ticks for conducting the assay, it is observed that larval based assay may not be the true alternative for AIT. For our experiments, we generated significantly high number of adults for the characterization of resistance to OP, SP and amidine compounds and AIT assay was found to be more satisfactory than larval based assay (Kumar et al., 2011, 2014; Ravindran et al., 2014).

In India, currently base line value for LC_{50} and LC_{95} of IVM has not been determined and so DC for characterization of field ticks has not been established. In the present study, experiments were conducted to determine the DC of IVM based on AIT using reference susceptible strain and to validate the DC against field collected ticks.

2. Materials and methods

2.1. Acaricide

Technical grade IVM (22, 23-dihydroavermectin B1, Sigma–Aldrich, USA) was used to prepare 4% primary stock solution in absolute ethanol with 2% Tween-20 (Eth-T 20). Two fold serial dilutions of primary stock in Eth-T20 (2%, 1%, 0.5%, 0.25% and 0.125%) were prepared as secondary stock solution and stored at −20 °C (Sabatini et al., 2001). Working concentrations were prepared in ultrapure water.

2.2. Reference tick line

The susceptible reference, IVRI-I line, was used as standard to establish LC_{50} and LC_{95} values. The reference tick strain (National registration no. NBAII/IVRI/BM/1/1998) was maintained in the Entomology Laboratory of the Indian Veterinary Research Institute. The susceptible status of the strain was monitored and the homogeneity amongst different generations was established by uniform biological parameters and by analyzing the sequences of 16S rDNA gene (Kumar Rinesh et al., 2011).

2.3. Determination of DC

The AIT was conducted according to the method of Sabatini et al. (2001) with minor modifications. Briefly, the pre-weighed engorged females of *R. microplus* (IVRI-I) were immersed in 10.00, 12.50, 25.00, 40.00, 50.00 and 80.00 ppm concentrations. Each concentration was replicated four times and ten adults were used per replication. The adult ticks were immersed for 30 min in a 100 ml conical flask and gently agitated in shaking incubator at 100 rpm at 25 °C. Ticks were removed from the flask, dried and kept in 5.5 × 1.5 cm plastic petri dishes. The petri dishes were kept in desiccators placed in BOD incubator maintained at 28 °C and 85 ± 5% RH for 14 days to allow egg laying. The control group of ticks were treated with ultrapure water and were maintained as above. Following parameters were compared:

(a) Mortality = engorged females that oviposited were considered as live and females that did not oviposit or oviposited small amount of black eggs (non viable) were considered as dead.
(b) Egg masses on day 14 post AIT
(c) Reproductive index (RI) = Egg masses (mg)/Engorged female weight (mg)
(d) % Inhibition of oviposition (IO) = RI control − RI treated/RI control X 100

2.4. Study area

A highly diverse area in respect to geographical distance, socio-economic pattern, animal population and husbandry practices was selected for the collection of ticks following two stage stratified random sampling method for the validation of DC. The climatic conditions of the regions are highly conducive for growth and development of TTBDs. Both organized and unorganized farms of different districts of northern and eastern region of India namely Lucknow (26.8° N, 80.9° E) (LKO), Kanpur (26.44° N, 80.33° E) (KAN), Amroha (28.9° N, 78.46° E) (AMR) and Mathura (27.49° N, 77.67° E) (MTH) of Uttar Pradesh (UP), Fatehgarh Sahib (30.38°N 76.23°E) (FTG) and Ludhiana (30.9°N 75.85°E) (LDH) of Punjab and Saharsa (25.88° N, 86.6° E) (SRS) of Bihar were selected for tick collection. According to the 19th animal census data, the total cattle population of UP, Punjab and Bihar states are 18.84, 4.93 and 28.04 million, respectively, with the estimated milk production of 5.937, 3.086 and 3.762 million tonnes, respectively (Basic Animal Husbandry and Fisheries Statistics, 2014). The use of IVM was reported to be increased in this part of the country during the last five to eight years.

2.5. Characterization of field isolates

The samples collected from a particular district were pooled, designated as an isolate and washed thoroughly in distilled water and labelled. For AIT, 25, 50, 100, 200 and 400 ppm concentration of IVM was prepared as mentioned above. The AIT was conducted at sample processing centre located in vicinity to the collection station when fully engorged female ticks were collected in a large numbers (more than 200–250). However, when number of collected ticks were not sufficient for conducting AIT, then the ticks were kept for laying eggs at optimum maintenance conditions of 28 °C and 85 ± 5% RH (Ghosh and Azhahianambi, 2007). The egg masses of each isolate were pooled separately in a glass tube closed with muslin cloth and kept in similar condition to hatch the larvae. The 10–14 days old unfed larvae were released on cross-breed calves for feeding to get sufficient number of fully engorged females for AIT. Separate calf was maintained for each isolate and the experimental calves were maintained in the large animal rearing facility of the division of Parasitology of the institute. After 18–20 days of feeding, the engorged females were collected and resistance/susceptibility to IVM was determined using a minimum 200–250 female ticks/isolate.