Veterinary Parasitology 216 (2016) 108–117

Contents lists available at ScienceDirect

Veterinary Parasitology

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

Research paper

Multiple mutations in the acetylcholinesterase 3 gene associated with organophosphate resistance in *Rhipicephalus* (*Boophilus*) *microplus* ticks from Punjab, India

Jyoti [a,*], Nirbhay Kumar Singh [a], Harkirat Singh [a], Niraj Kumar Singh [b], Shitanshu S. Rath [a]

[a] Department of Veterinary Parasitology, College of Veterinary Science, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana 141004, India
[b] School of Animal Biotechnology, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana 141004, India

ARTICLE INFO

Article history:
Received 17 July 2015
Received in revised form
14 November 2015
Accepted 11 December 2015

Keywords:
Acetylcholinesterase
India
OP resistance
Punjab
Rhipicephalus (*Boophilus*) *microplus*

ABSTRACT

The organophosphate (OP) resistance status in *Rhipicephalus* (*Boophilus*) *microplus* ticks collected from seventeen districts located in the northwestern Indian state, Punjab were characterized using three data sets (bioassay, biochemical and molecular assays). Adult immersion test (AIT) was adopted and the resistance factors (RF) for the field isolates were determined. Resistance to malathion was detected in 12 isolates among which 11 showed level I resistance status while level II status was recorded in one isolate (RF of 5.35). To understand the possible mechanism of resistance development, acetylcholinesterase (AChE) activity and gene sequences of the AChE3 were analyzed. A significantly ($P < 0.001$) higher level of percent uninhibited AChE activity was recorded in all field isolates (36.36 ± 0.46–43.77 ± 1.21) in comparison to the susceptible population (29.39 ± 0.40). The AChE activity was positively correlated with RF against malathion with a correlation coefficient (r) of 0.359. Analysis of nucleotides and their deduced amino acids sequences of partial AChE3 gene revealed the presence of six amino acid substitutions (I48L, I54V, V71A, I77M, S79P and R86Q). Three novel amino acid substitutions (V71A, I77M and S79P) in partial AChE3 gene were also identified in some of the isolates which may possibly have a role in OP resistance development. The PCR-RFLP assay with *Hae*III revealed the presence of restriction site corresponding to R86Q mutation in all the field isolates along with an additional restriction site in seven field isolates corresponding to V71A mutation. The results of the study indicate the involvement of both insensitive AChE and higher percent uninhibited AChE activity as the possible mechanism in these field isolates.

1. Introduction

The one-host cattle tick, *Rhipicephalus* (*Boophilus*) *microplus* has a wide geographic distribution, spanning tropical and subtropical regions between parallels 32°N latitude and 35°S latitude, including countries in Latin America, Africa, Asia and Oceania (Wharton et al., 1970). It is considered as the most important ecto-parasite in the cattle industry because of the direct and indirect damage that it causes to animal health, which considerably limits bovine productivity (Maza et al., 2013). The economic impact of ticks and tick borne diseases (TTBDs) on the Indian cattle industry was estimated at 498.7 million US$ annually (Minjauw and McLeod, 2003). In India, about 60% of livestock is reared by small and marginal farmers and use of various organophosphate (OP) compounds is very common for the control of livestock and poultry pests (Ghosh et al., 2006). Organophosphate compounds are also used against agriculturally important pests and for mass eradication of mosquito larvae in their breeding places (ICMR Bulletin, 2002). Resistance to OP compounds in *R.* (*B.*) *microplus* has become widespread throughout the world (Miller et al., 2005; Mendes et al., 2007; Baffi et al., 2008) including India (Rath et al., 2006; Kumar et al., 2011; Jyoti et al., 2014).

The state of Punjab has been one the front runners in white revolution in the country and is 5th in milk production amongt all the Indian states with cattle and buffalo population of 2.427 and 5.159 million, respectively (DAHD, 2014). *R.* (*B.*) *microplus* is the most prevalent tick infesting all age groups of domestic livestock in various agro-climatic zones of Punjab state, India (Haque et al., 2011; Singh and Rath, 2013). Recent studies report development of resistance against several acaricides in *R.* (*B.*) *microplus* from the Punjab state (Singh et al., 2010, 2014; Singh and Rath, 2014; Jyoti et al., 2014; Nandi et al., 2015). Therefore, to protect the

* Corresponding author. Fax: +91 16102400822.
E-mail address: jyoti76vet@gmail.com (Jyoti).

http://dx.doi.org/10.1016/j.vetpar.2015.12.004
0304-4017/

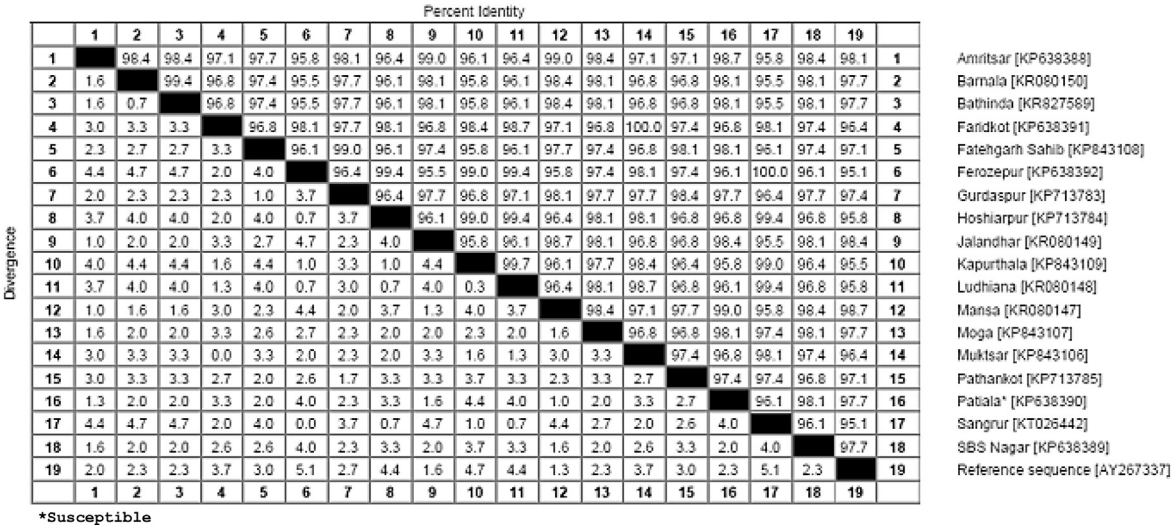

Fig. 1. Sequence pair distances of partial AChE3 gene of various field isolates of *R.* (*B.*) *microplus*.

huge animal population of the state from ticks, monitoring of acaricide resistance is crucial to slow down the process of spreading resistance and to develop region specific strategy for tick control. Reliable detection of resistance is a major component of acaricide resistance monitoring system. Traditionally, conventional bioassays have been the primary diagnostic tools however, the advent of molecular and biochemical techniques have paved ways for sensitive early detection and understanding the possible mechanisms of resistance development.

Acetylcholinesterase (AChE) is the target for OP acaricides (O'Brien, 1967), and AChE insensitivity was reported as the primary mechanism of OP-resistance in *R.* (*B.*) *microplus* (Lee and Batham, 1966; Li et al., 2003; Temeyer et al., 2004, 2007, 2009, 2013), although metabolic detoxification may also be important (Li et al., 2005; Baffi et al., 2008). Previous studies identified three *R.* (*B.*) *microplus* genes presumptively encoding AChEs, BmAChE1 (Baxter and Barker, 1998), BmAChE2 (Hernandez et al., 1999; Baxter and Barker, 2002) and BmAChE3 (Temeyer et al., 2004). Searches for mutations associated with OP-resistance in BmAChE1 and BmAChE2 failed to reveal a clear association between any identified amino acid substitutions. The R86Q substitution in BmAChE3 was the first mutation in ticks demonstrated to confer insensitivity to OP, resulting in approximately 20-fold reduction in paraoxon sensitivity (Temeyer et al., 2007).

In the present study, *R.* (*B.*) *microplus* populations collected from seventeen districts of the Punjab state, India were characterized using bioassay, biochemical and molecular methods to assess the resistance status to OP compound. The alterations in the levels of percent uninhibited AChE activity and mutations identified in partial AChE3 gene were correlated with phenotypic resistance to OP acaricide to generate information regarding the possible mode of development of resistance with an aim to develop strategy to control the problem in sustainable manner.

Table 1
The LC_{50}, LC_{95}, resistance factor and resistance level against malathion of field isolates of *R.* (*B.*) *microplus* and their percent uninhibited acetylcholinesterase (AChE) activity.

Tick isolates	Mortality	LC_{50} (ppm) (95% CL)	LC_{95} (ppm) (95% CL)	RF[a] (RL[b])	AChE (% uninhibited activity)		
					Mean ± SE	*P* value	ER[c]
Amritsar	4.71 ± 0.71	3099.5 (2980.2–3223.4)	6914.8 (6343.8–7537.1)	1.85 (I)	38.07 ± 1.17	<0.001	1.295
Barnala	1.58 ± 0.21	3509.4 (3081.1–3997.2)	38317.9 (29205.7–50273.1)	2.09 (I)	43.61 ± 1.18	<0.001	1.483
Bathinda	2.86 ± 0.49	2276.9 (2118.0–2447.7)	8526.5 (7363.1–9873.6)	1.36 (S)	38.65 ± 1.26	<0.001	1.315
Faridkot	3.63 ± 0.52	2225.1 (2102.7–2354.6)	6288.9 (5610.1–7049.9)	1.33 (S)	36.36 ± 0.46	<0.001	1.237
Fatehgarh Sahib	4.79 ± 0.71	3541.6 (3392.3–3697.4)	7782.6 (7139.9–8483.0)	2.11 (I)	38.37 ± 0.67	<0.001	1.305
Ferozepur	2.55 ± 0.44	6920.5 (6407.8–7474.1)	30618.0 (26169.3–35823.1)	4.12 (I)	42.02 ± 1.27	<0.001	1.429
Gurdaspur	3.95 ± 0.64	4267.9 (4053.1–4494.1)	11077.1 (9979.4–12295.6)	2.54 (I)	38.33 ± 1.65	<0.001	1.304
Hoshiarpur	3.07 ± 0.77	3391.8 (3172.8–3625.8)	11590.6 (10113.9–13282.8)	2.02 (I)	36.78 ± 1.12	<0.001	1.251
Jalandhar	3.69 ± 0.31	3436.8 (3251.4–3632.6)	9555.0 (8208.8–11122.0)	2.05 (I)	38.46 ± 0.93	<0.001	1.308
Kapurthala	2.87 ± 0.55	1342.8 (1254.9–1436.7)	5012.3 (4358.4–5764.1)	0.80 (S)	37.24 ± 0.55	<0.01	1.267
Ludhiana	2.26 ± 0.56	1554.5 (1419.6–1702.2)	8265.3 (6853.6–9967.9)	0.93 (S)	38.88 ± 0.90	<0.001	1.322
Mansa	1.60 ± 0.27	2992.1 (2631.6–3402.1)	31554.7 (24124.3–41273.5)	1.78 (I)	41.24 ± 1.41	<0.001	1.403
Moga	2.47 ± 0.27	8975.8 (8257.4–9756.7)	41352.8 (34867.4–49044.4)	5.35 (II)	40.42 ± 1.02	<0.001	1.375
Muktsar	1.01 ± 0.36	1002.3 (821.5–1222.8)	41376.0 (26780.6–63925.9)	0.60 (S)	43.77 ± 1.21	<0.001	1.489
Pathankot	3.24 ± 0.87	4158.3 (3904.5–4428.6)	13309.2 (11705.6–15132.6)	2.48 (I)	37.24 ± 1.27	<0.001	1.267
Sangrur	2.76 ± 0.66	4949.9 (4595.9–5331.0)	19473.5 (16715.4–22686.6)	2.95 (I)	40.37 ± 0.75	<0.001	1.373
SBS Nagar	2.51 ± 0.47	6955.9 (6411.0–7547.2)	31276.8 (26438.5–37000.5)	4.14 (I)	43.97 ± 0.81	<0.001	1.496
Patiala[d]	3.60 ± 1.05	1678.8 (1585.3–1777.8)	4792.4 (4278.9–5367.5)	1.0 (S)	29.39 ± 0.40	–	1

[a] RF: LC_{50} of field population/LC_{50} of susceptible (Patiala) isolate.
[b] Level of resistance; susceptible (S) (RF < 1.5), level I (RF = 1.5–5.0), level II (RF = 5.1–25.0), level III (RF = 25.1–40) and level IV (RF > 40.1).
[c] Enzyme ratio.
[d] Susceptible isolate.