

Downregulation of Carboxylesterase Gene Expression In *Hyalomma Anatolicum* Ticks Exposed to Cypermethrin And Deltamethrin

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ABSTRACT

Acaricide resistance (AR) is a major challenging tick control issue, a process by which the spread of tick-borne diseases, such as Crimean-Congo hemorrhagic fever (CCHF) can easily be stopped. The AR can be performed by ticks deploying some enzymes, such as carboxylesterases (CXEs) that degrades the these acaricide into harmless metabolites to the host tick. The current study was performed to understand the gene expression status of the CXE gene after experimental tick exposure to cypermethrin and deltamethrin. The study included the use of 40 adult female ticks divided into 20 ticks per acaricide used (10 exposure and 10 controls). The ticks exposed to each acaricide as spraying in plates and were let without exposure for 10hrs until total RNA was extracted from all tick. The pooled RNA from each group was subjected to a quantitative real-time PCR (qRT-PCR) method. The results revealed significant ($p<0.05$) decreases in the mRNA expression level of the CXE gene after the exposure to the acaricides. The presented study, here, may indicate important information regarding susceptibility of ticks to cypermethrin and deltamethrin with no observed resistance.

Introduction

Ticks are ectoparasites that feed on blood and are found worldwide, particularly in tropical and subtropical areas. They infest terrestrial and semi-terrestrial vertebrates. Ticks also function as reservoirs for the transmission of infectious agents within their hosts. The livestock industry experiences considerable economic losses because of host blood exhaustion, overall discomfort, irritation, decreased milk products and meat, compromised immune functions, and destruction of hides, among various other adverse effects (1–5).

Prior to the the arrival of synthetic acaricides, various substances including cotton-seed oil, beaumont crude oil, and combinations of lard oil with sulfur or kerosene oil were employed topically on body surface for the purpose of managing tick infestations. The organophosphates (OPs), macrocyclic lactones (MLs), and synthetic pyrethroids (SPs) are all frequently employed acaricides that exert their effects on the central nervous system of ticks via different pathways (6–8). These mechanisms involve some modulation, such as gamma-aminobutyric acid (GABA)-gated chloride channels, inhibition of acetylcholine-esterase (AChEs), and targeting of voltage-gated sodium channels. Various acaricides exhibit distinct target specificity and diverse mechanisms of action, thereby influencing the reproductive capacity, growth, and overall survival of different species of tick. Various methods can be employed to administer acaricides onto host animals, including spraying, washing, pouring, and injections (9–11).

Tick AR refers to the heritable characteristics exhibited by a population of ticks, which have been specifically chosen due to their interaction with an acaricide. According to Rodriguez-Vivas et al. (2018), there is a notable increase in the survival rate of ticks following their exposure to a particular concentration of acaricides. The mutated genes, which have been passed down from the ticks that have survived, are initially infrequent and seldom observed within the tick population. However, over time, their prevalence gradually rises. Acquired resistance can be defined as a form of resistance that arises due to heritable decreases in the susceptibility of a drug over time. Consequently, this leads to the development of phenotypic resistance (12–14). The concept of tolerance pertains to the capacity of a parasite to endure and persist in the presence of a particular dosage of medication that is typically deemed efficacious. The phenomenon of resistance occurring across various active chemical ingredients that share similar mechanisms of action is commonly referred to as cross-resistance (15–20). There are typically three primary forms of AR that are widely recognized. Metabolic resistance is attained through the process of acaricide detoxification, which involves the enzymatic activity of cytochrome P-450s (CYP450), esterases, and glutathione S-transferase (GST). The phenomenon known as "target site modification resistance" refers to the development of AR in neuronal enzymes and receptors (21,22).

The primary factors that accelerate the selection of acaricide resistance in ticks include inaccurate dilution, insufficient application, continuous utilization, and overdosing. Some species may exhibit a higher susceptibility to the development of AR, as a result of the supportive conditions facilitated by their extensive distribution and/or unique characteristics of their life cycle (23–27).

AR is a major challenging tick control issue, a process by which the spread of tick-borne diseases, such as CCHF can easily be stopped. The AR can be performed by ticks deploying some enzymes, such as CXEs that degrades the these acaricide into harmless metabolites to the host tick. The current study was performed to understand the gene expression status of the CXE gene after experimental tick exposure to cypermethrin and deltamethrin.

Materials methods

Samples

The study included the use of 40 adult female ticks divided into 20 ticks per acaricide used (10 exposure and 10 controls). The ticks exposed to each acaricide as spraying in plates and were let without exposure for 10hrs until total RNA was extracted from all tick.

Extraction of total tick RNA

The tick total RNA was extracted using the AddBIO kit (AddBIO, Korea) and depending

on the protocol accompanied the kit. In a brief, the ticks were placed in liquid nitrogen for crushing and then placed in a lysis buffer. Then, the remaining steps were followed. The resulted RNA was measured using Quantus™ Fluorometer (Promega, USA). The RNA was kept in -80°C for performing the qRT-PCR on pooled RNA from each group.

Synthesis of cDNA

Using the AddBIO kit, the cDNA was produced by the following; 3µl, 10µl, 2µl, 1µl, and 4µl (100ng) in a total volume of 20µl for H₂O, 2X add script cDNA, dNTPs, random oligos hexamer, and RNA, respectively. In a thermocycler, the conditions were (25°C-10mins), (50°C-60mins), and (80°C-5mins) for the priming, reverse transcriptase (RT), and RT inactivation, respectively.

QRT-PCR

The AddScript RT-qPCR Syber master (AddBio, Korea) was used to perform qRT-PCR with the application of the kit instructional steps. Employing 4µl, 10µl, 2µl, and 2µl in 20µl total reaction volume for the H₂O, AddScript RT-qPCR, (0.05pmol/20µl) each primer direction (Table 1), and cDNA, respectively.

Table 1: Primers used (designed in the current study)

Primer name		Sequence '5-----3'	Accession number
Carboxylesterase	Carbo-F	AGCATCGACCTCTCGTCCAAC	JX392019.1
	Carbo-R	GTCGGCATACTTGTCTTCGATG	
GAPDH (Housekeeping)	GAB-F	AGGCTCAGCAGCACATTGAT	KU248453.1
	GAB-R	ATGCCGAAGTTGTCGTGGAT	

The thermocycler BioRad (USA) conditions were 50°C-2mins, 95°C-10mins, 95°C-15s, 57°C-30s, 72°C-30s, 95°C-15s, 60°C-60s, and +0.3°C of 95°C-15s for a one-repeat activation, a one-repeat initial denaturation, 40X of (denaturation, annealing, and extension), a one-repeat melting analysis, a one-repeat melting analysis, and a one-repeat melting analysis, respectively. A normalization step was done on the RNA using the $2^{-\Delta\Delta CT}$ method developed by Schmittgen and Livak, (2008) (28).

Results

The results revealed significant ($p<0.05$) decreases in the mRNA expression level of the CXE gene after the exposure to the acaricides in a comparison with that from the control group (Figure 1). The fold changes for the mRNA of the CXE gene related to the cypermethrin and deltamethrin were (0.4 and 0.5). In the case of the control group, the fold change was (1.5).

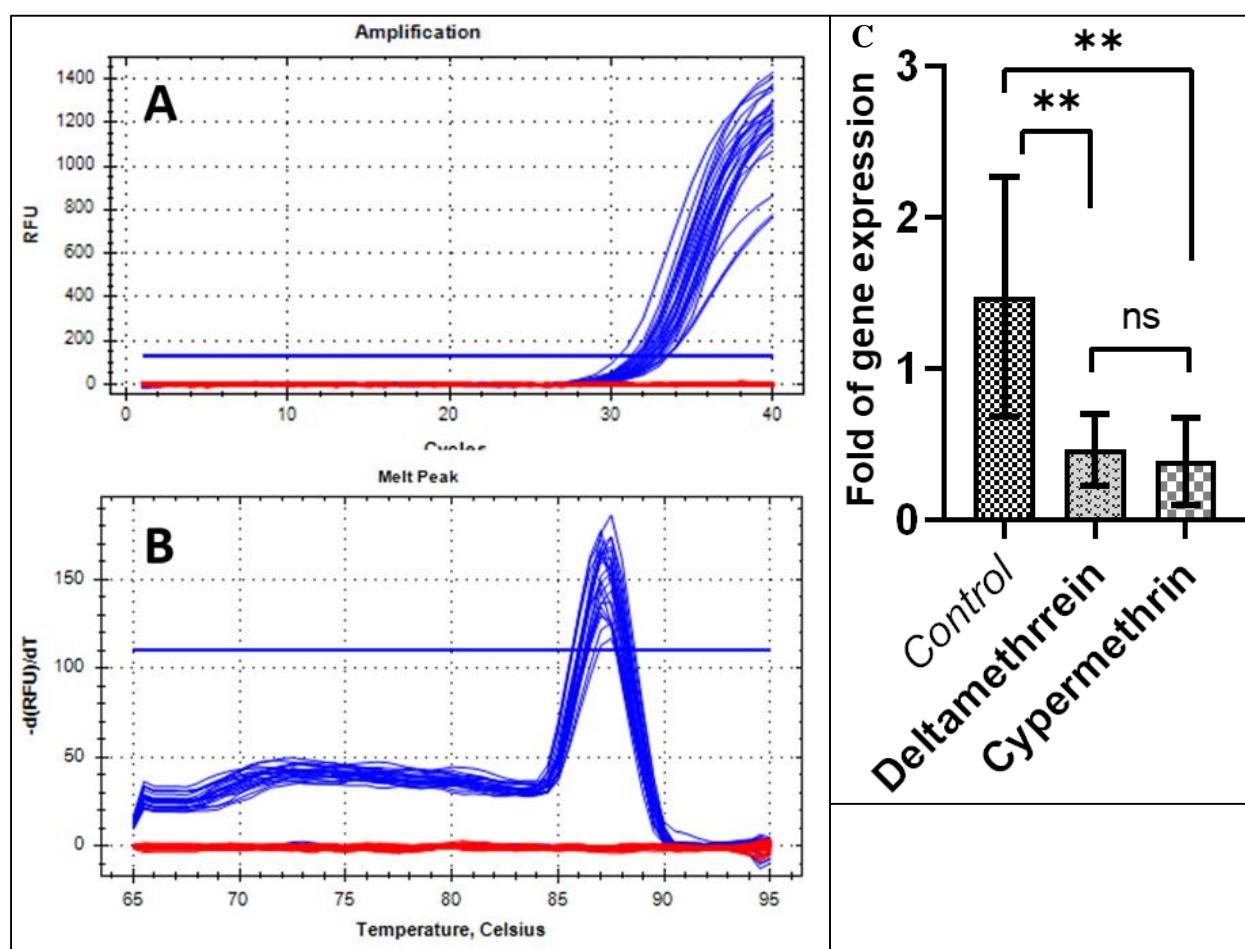


Figure 1: Carboxylesterase gene mRNA expression from tick exposed to cypermethrin and deltamethrin. **A.** Amplification curve. **B.** Melting curve. **C.** Fold change of carboxylesterase gene expression.

Discussion

The development of resistance to acaricides has significantly impeded the ability of livestock farmers to effectively handle and control ticks and tick-borne diseases. The global research community has been actively investigating the emergence of AR in ticks. This issue has garnered significant attention due to the challenges and costs associated with the development of novel acaricides and the breeding of cattle that are resistant to ticks. The optimal approach for evaluating AR in tick populations involves assessing its efficacy against ticks. Nevertheless, laboratory bioassays conducted in vitro offer valuable insights into the occurrence of acaricide resistance development. Numerous in-vitro studies have been conducted globally to investigate the characterization of AR in tick communities (29–33).

The initial step in obtaining phenotypic data on the extent of tick resistance involves the utilization of bioassay methods to characterize AR in communities of ticks that have displayed AR. Most reports discussing the emergence of phenotypic AR in tick communities globally focus

on *Rhipicephalus (Boophilus) microplus*. In the study conducted, a total of 3939 tick populations were examined to assess the emergence of resistance. Out of these populations, 3391 (86%) were identified as *R. (B) microplus*. The analysis revealed a global combined incidence estimation of AR development in these ticks to be 66.2% (34–36).

The development of AR on a global scale has exhibited a higher rate in these ticks. Nevertheless, a decreased prevalence of AR was noted in the following tick species: *R. annulatus* and *H. anatolicum*. *R. (B) microplus* and *R. (B) decoloratus* are ticks that exclusively infest a single host. The accelerated development of AR in these ticks have been linked to their shorter life cycle and greater rate of reproduction, necessitating frequent administration of acaricides. The repeated exposure of substantial amounts of the tick community to acaricides contributes to the emergence and dissemination of resistant ticks, thereby facilitating the evolution and spread of AR (37,38). It is worth noting that AR is typically infrequent in ticks that have multiple hosts, as these ticks exhibit a broad range of hosts encompassing both domestic and wild animals. Furthermore, it is commonly observed that they exhibit extended life cycles, wherein the duration of the parasitic stage is typically shorter than the intervals between treatments commonly employed by agricultural practitioners. Consequently, a greater percentage of these ticks typically evade acaricide treatment, known as refugia, leading to a diminished level of selection pressure for the development of AR (39). Numerous countries, such as Benin, Brazil, Mexico, and India have documented instances of heightened resistance to synthetic compounds, such as deltamethrin and cypermethrin within populations of ticks (40,41).

Conclusion

The presented study, here, may indicate important information regarding susceptibility of ticks to cypermethrin and deltamethrin with no observed resistance.

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