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Short communication

C190A knockdown mutation in sodium channel domain II of pyrethroid-resistant *Rhipicephalus appendiculatus*

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ABSTRACT

This study investigated the target site mutations in the partial sequence of voltage-sensitive sodium channel (VSSC) domain II of synthetic pyrethroid (SP)-resistant *Rhipicephalus appendiculatus*. Genomic DNA was extracted from seven tick populations (two susceptible and five resistant) collected from central, eastern and southwestern Uganda. The PCR amplicons of the VSSC domain II were cloned and sequenced to determine novel single nucleotide polymorphisms (SNP). A non-synonymous mutation C78 A corresponding to C190 A was found in all the five SP-resistant ticks. The C78 A mutation led to amino acid substitution from leucine to isoleucine (L21I) which was previously reported to confer knockdown (*kdr*) mutation in *R.* (*Boophilus*) *microplus*. The genetic confirmation of SP-resistant *R. appendiculatus* in central and southwestern Uganda calls for an urgent strategy for controlling the ticks.

1. Introduction

Ticks are one of the leading vectors of livestock diseases in the tropics (Jongejan and Uilenberg, 2004). In Africa, ticks transmit economically important pathogens such as *Theileria* spp., *Anaplasma* spp. and *Babesia* spp. to domestic animals (Adjou Moumouni et al., 2015; Makala et al., 2003). The economic losses associated with ticks and tick-borne diseases (TBD) in Africa has been widely documented (Mukhebi and Perry, 1992; Mukhebi et al., 1999; Ocaido et al., 2009) although undervalued (Jongejan and Uilenberg, 2004). *Rhipicephalus appendiculatus* is a vector for *Theileria parva* that causes East coast fever (ECF) in cattle in eastern, central and parts of southern Africa (Gilioli et al., 2009; Kalume et al., 2011; Kanduma et al., 2016; Perry et al., 1991). East coast fever is an acute and fatal disease of calves and exotic cattle in Uganda (Mugisha et al., 2005; Ocaido et al., 2009).

Acaricides such as synthetic pyrethroids, organophosphates and amidines are used for control of ticks to prevent TBD (Mugabi et al., 2009; Okello-Onen et al., 1998). However, a recent report indicated that there are pyrethroid-resistant *R. appendiculatus* ticks in central and southwestern Uganda (Vudriko et al., 2016). Of serious concern was that the majority of those *R. appendiculatus* ticks showed 100% survival after treatment with twice the discriminating concentration (0.1 mg/ml) of cypermethrin and deltamethrin (Vudriko et al., 2016). Previous studies have associated stable pyrethroid resistance in subgenus *Boophilus* ticks and insects such as *Drosophila* and the house fly (*Musca domestica*) to target site mutations in the voltage-sensitive sodium channel (VSSC) (Dong, 2007; Abbas et al., 2014; Guerrero et al., 2012). Both knockdown (*kdr*) and super *kdr* in VSSC were reported as the main mechanism of SP resistance in *R.* (*B.*) *microplus* (He et al., 1999; Lovis et al., 2012; Stone et al., 2014; van Wyk et al., 2016) and *R.* (*B.*)

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Table 1
The district of origin and susceptibility of tick populations used in this study against synthetic pyrethroids (cypermethrin and deltamethrin).

Region	District	Year of tick collection	Tick ID	Resistance status [a]	
				Cypermethrin	Deltamethrin
Northwest	Adjumani	2015	AI6[b]	S	S
Central	Kampala	2014	C1	R	R
	Wakiso	2014	C2	R	R
	Ssembabule	2014	W21	R	R
Southwest	Mbarara	2014	W4	R	R
	Rukungiri	2015	W18	S	S
		2015	W19	R	R

[a] Determined by larval packet test at discriminating dose (0.05 mg/ml).
[b] Susceptible reference population (100% mortality); S, susceptible (> 97% mortality); R, Resistant tick population (0–12% mortality). Susceptibility of tick populations of central and southwestern regions were determined in an earlier study (Vudriko et al., 2016).

decoloratus (Vudriko et al., 2017a). To the best of our knowledge, target site mutations in SP-resistant *R. appendiculatus* have not yet been reported. In the present study, we investigated novel mutations in the VSSC domain II of highly SP-resistant *R. appendiculatus*.

2. Materials and methods

2.1. Tick populations

A total of seven *R. appendiculatus* tick populations collected from six districts in northwestern (1), central (2) and southwestern (4) Uganda in our earlier study (Vudriko et al., 2016) were used for the study. The susceptibility of the ticks against discriminating dose (DD) and 2 × DD of the SP cypermethrin and deltamethrin were determined by larval packet test (Vudriko et al., 2016). Two of the tick populations were susceptible and five were resistant to discriminating dose of both SP (Table 1).

2.2. Extraction of genomic DNA and amplification of VSSC domain II

Genomic DNA was extracted from 30 pooled larvae with NucleoSpin Tissue® DNA extraction kit (Macherey-Nagel, German) following manufacturers recommendation. PCR amplification was carried out using the primer pair RmNaDIIF1 (5′-TACGTGTGTTCAAGCTAGCCAA-3′) and RmNaDIIR1 (5′-ACTTTCTTCGTAGTTCTTGCCAA-3) (Stone et al., 2014) and 0.014 U of KOD FX Neo plus (Toyobo, Japan) with Veriti Thermal Cycler (Applied Biosystems, USA). The amplicons were electrophoresed in 1.5% agarose gels, stained with ethidium bromide and visualized under UV lamp.

2.3. Cloning of amplified VSSC domain II genes and bioinformatics analysis

The VSSC domain II amplicons were purified using Wizard® SV Gel and PCR Clean-up System (Promega, USA) following the manufacturer's instruction. The TA cloning was carried using pGEM-T easy ligation kit (Promega, USA) and ECOS™competent *Escherichia coli* DH5α (Nippon gene, Japan). Ligation, transformation and sequencing were carried out as described previously (Vudriko et al., 2017a). The SP6 and T7 promotor primers were used for sequencing with BigDye v3.1 Terminator Cycle Sequencing Kit and the 3730×l DNA Analyzer (Applied Biosystem, USA). The resultant nucleotide sequences were compared with sequences in GenBank using BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi). An online tool EMBOSS Transeq (http://www.ebi.ac.uk/Tools/st/emboss_transeq/) was used for translating the nucleotide sequences to amino acid sequence for each tick population. Multiple sequence alignment was done using BioEdit version 7.2.5 (Tom Hall Ibis Biosciences, CA) to determine SNP and amino acid substitutions in the VSSC domain II.

The resultant *R. appendiculatus* VSSC domain II nucleotide sequences from this study were deposited in GenBank under the accession numbers MH053429-MH053435 (Table S1).

2.4. Ethical considerations

This study was approved by the College of Veterinary Medicine, Animal Resources and Biosecurity, Makerere University (No. VAB/REC/15/104). The DNA experiment was carried out according to ethical guidelines for the use of DNA samples permitted by Obihiro University of Agriculture and Veterinary Medicine under approval number 1705.

3. Results and discussion

The partial VSCC domain II nucleotide sequence for the susceptible *R. appendiculatus* ticks (AI06) was identical to those of the susceptible reference *R.* (*B.*) *decoloratus* (accession# KY659478) and *R.* (*B.*) *microplus* (accession# AF134216). This suggests that VSSC domain II is highly conserved among the three Rhipicephaline ticks as postulated in our earlier report (Vudriko et al., 2017a). Two mutations were

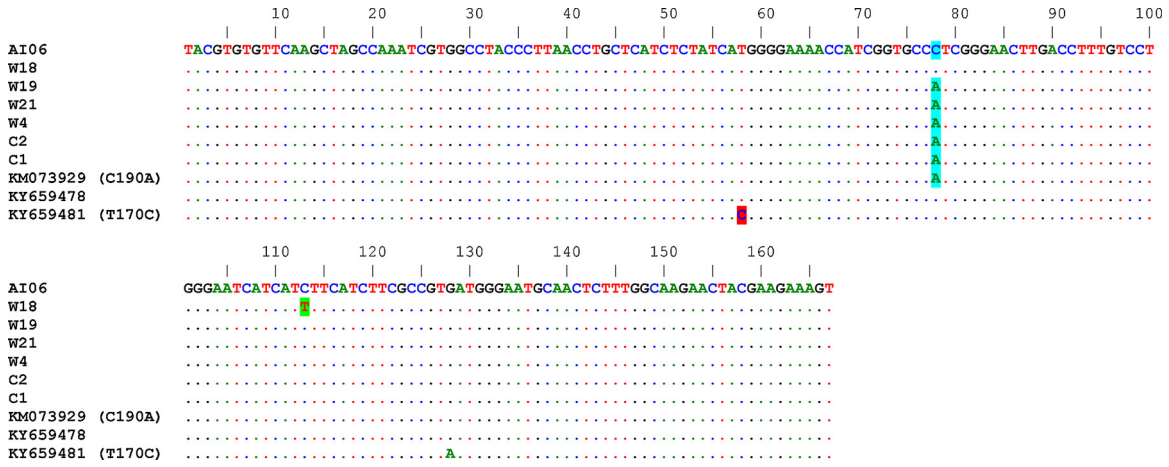

Fig. 1. Multiple nucleotide sequence analysis of VSSC domain II for SP-susceptible and -resistant *R. appendiculatus* ticks.
Accession# KM073929 C190 A, SP-resistant *R.* (*B.*) *microplus* VSSC domain II with C78 A/ C190 A *kdr* mutation (highlighted blue); Accession# KY659478, VSSC domain II for SP-susceptible reference *R.* (*B.*) *decoloratus* tick from Uganda; Accession# KY659481, VSSC domain for II SP-resistant *R.* (*B.*) *decoloratus* with super *kdr* T170C mutation (highlighted red).

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