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Evaluation on Diazinol Resistance of Economically Important Dairy Cattle Ticks in Central Highlands of Ethiopia

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Abstract: Tick infestation is an economically important condition in cattle. In addition to increasing susceptibility to other diseases, skin damages inflicted by ticks downgrade the quality of skin and hide and the production of milk and wool. Control of these parasites remains a challenge due to the presence of resistance to commercial acaricides. A cross-sectional study using a questionnaire survey followed by an in-vitro acaricidal efficacy test was conducted from 2014 to 2017 to check the existence of resistant tick species to chemical acaricide. Most cases of tick infestation (94.6%) were treated by owners following veterinary professionals' advice (69.4%). *Amblyomma variegatum (A. variegatum), Boophilus decoloratus (B. decoloratus)* and *Rhipicephalus evertsi evertsi (R. evertsi evertsi)* test and reference engorged female ticks were subjected to a modified larval packet test (LPT). Except for the mortality rate (3.33%) in the control group, both test and reference treatment groups exhibited a higher mortality rate (90% to 100%) at all tested concentration levels. A significant variance (P=0.0001) proofed by statistics was detected among test tick species. The LC_{50} of Diazinol against *A. variegatum, B. decoloratus, R. evertsi evertsi* and for their references was 0.03834, 0.0783, and 0.03834, respectively. *A. variegatum* and *R. evertsi evertsi* showed a resistance factor of 1.310, while *B. decoloratus* exhibited a factor of 2.676 indicating a level 1 resistance for Diazinol. Further investigation on the existence and seasonal dynamics of other tick species should be investigated. Future works should also look at the utilization of acaricides in a wider area of the district and their efficacy against the existing tick species. Furthermore, farmers' awareness on the utilization of acaricides should be enhanced through training.

Keywords: Acaricide Resistance, Adea Berga, *A. variegatum*, *B. decoloratus*, Dairy Cattle, *R. evertsi evertsi*, Ticks

1. Introduction

Ticks are the most common ectoparasites of wild and domestic animals worldwide [1]. Ticks remain an economically important parasites affecting the production and productivity of farm animals. Many of protozoal, bacterial, rickettsial and viral diseases are spread by ticks as compared to other group of arthropods [2]. In addition to increasing susceptibility to other diseases, skin damages inflicted by ticks downgrade the quality of skin and hide and the production of milk and wool [3]. Even though there are several commercial acaricides, tick control vestiges a challenge for several countries in the world [4]. The use of insecticides is still the basic means for the control of most ectoparasites [5]. More than 50 different species of ticks are reported to exist in Ethiopia. Of which, *A. variegatum*, *A.*

cohaerens, A. lepidum, A. gemma, B. decoloratus, R. bergeoni, R. evertsi evertsi, R. pulchellus, Hyalomma marginalis rufipes and Hyalomma dromedari are the most frugally important ones [6]. Tick control is mainly performed by means of commercially available acaricides which includes organochlorines, organophosphates, carbamates, amidines or synthetic pyrethroids [7]. However, due improper dosage and frequent use of these chemical acaricides, resistance is being developed in many countries [8] including Ethiopia [9]. *B. decoloratus* strains showed a high degree of resistance to Dieldrine, Diazinon, Chlorfenvinphos and Coumaphos in the study conducted at Holota, East Shewa Zone of Oromiya regional state Ethiopia. Far-reaching use of acaricides like Bacdip; uneven spraying, failure to keep suitable lethal concentrations and other managerial limitations are reported to decrease the fruitful use of acaricides and increase the prospect of developing acaricide resistance [10]. Hence unceasing studies on the resistance level of the tick population in areas like Adea Berga where previous studies did not conduct is highly compulsory to carryout proficient tick control and/or tick burden reduction. The current study was conducted to evaluate the existence of acaricide resistant tick species of dairy cattle and optimize alternative tick control strategies.

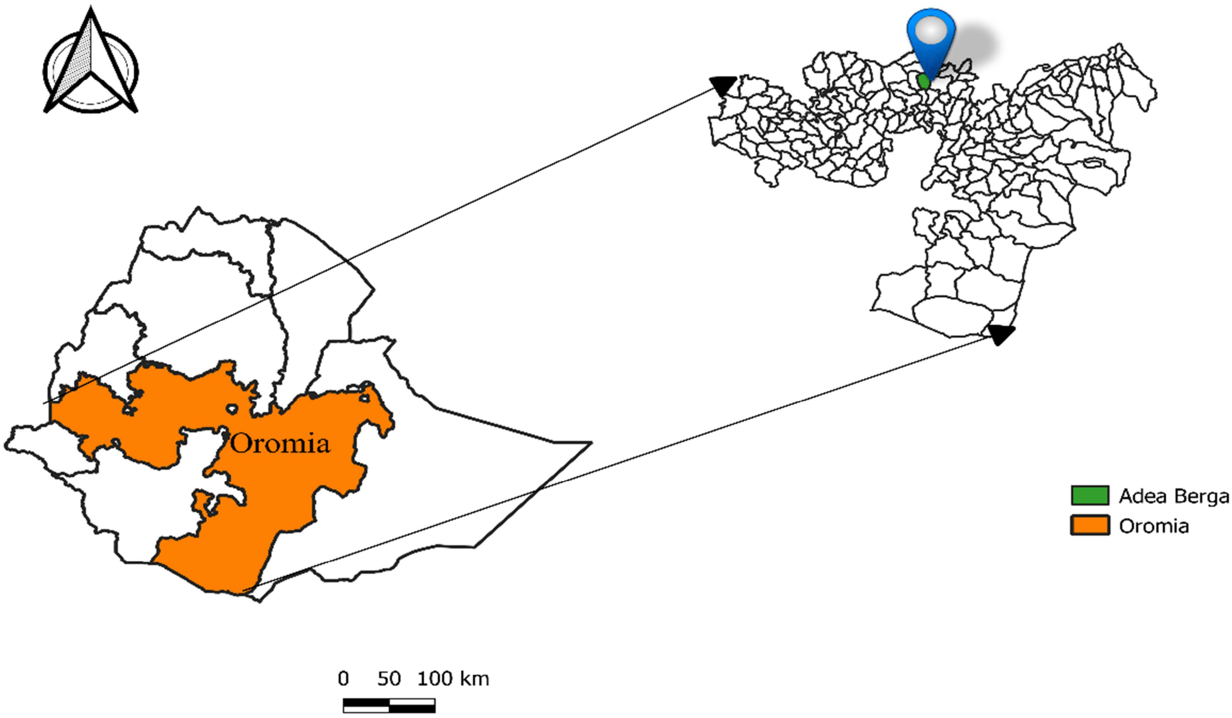


Figure 1. *Map of the study area.*

2. Materials and Methods

2.1. Study Area and Farming System

The study was performed in three Kebeles (lower administrative areas) called Chancho, Mugar and Agota which are found in Adea Berga district, West Shewa Zone of Oromiya Regional state, Ethiopia; located at 9^0 16'N latitude and 38^0 23'E longitude and at elevation ranging from 1166 to 3238 masl (meters above sea level). In this district, the rainfall pattern is bimodal, with a short rainy season from March to May and a long rainy season from June to September and the rest of the months are dry. The annual temperature and rainfall range from 18°C to 24°C and 1000 to 1225 mm, respectively. Farming is generally dominated by a smallholder crop-livestock mixed system in which cattle husbandry mainly stands for milk production and traction. The predominant cattle breeds are Zebu with some European crosses. Cattle are grazed during the daytime and very few are supplemented with available crop residues in the evenings. Tick infestation is one of the health constraints in the area and control relay on chemical acaricide which is often carried out when engorged adult ticks are appearing in large number on cattle.

2.2. Study Design

A questionnaire-based cross-sectional study design was conducted. Acaricide efficacy test was accompanied using larval packet test (LPT) following randomized complete block design, with 3 replication and 5-level of concentration. Larvae of *A. variegatum, B. decoloratus and R. evertsi evertsi* were challenged by different concentrations of Diazinol to fix the 50% lethal concentration (LC_{50}).

2.3. Questionnaire Survey

The survey part was conducted using a structured questionnaire. It contains basic quantitative and qualitative questions on the profile of farmers' and their animals, the occurrence of tick infestation and their distribution, control method of ticks and source of chemical acaricide as well as utilization of acarcides and their efficacy. The questionnaire was pre-tested and administered well ahead on 260 farmers purposively selected.

2.4. Study Animals

A total of 12 local dairy cattle were randomly selected for test tick collection from Chancho and Mugar Kebeles where tick infestation and use of chemical acaricide is relatively higher while reference ticks were collected from 10 local dairy cattle, located in Agota Kebele where the usage of acaricides was confirmed to be least or nil. No specific attention was given to the sex, age and other traits of the animals during selection.

2.5. Study Period

The study was conducted from 2014 to 2017.

2.6. Collection and Preparation of Ticks for Experiment

Adult engorged female test ticks were collected from Chancho and Mugar Kebeles while reference (not previously exposed to commercial acaricides) engorged adult female ticks were collected from Agota Kebele. All ticks were collected using hands from different body parts of local breed dairy cattle. Collected ticks were retained independently in different plastic bottles taped by gauze and labelled with time, date, place of collection and identification code and then transported to the Animal Health Research laboratory of National Agricultural Biotechnology Research Center where tick identification, rearing and an in *vitro* acaricidal test was performed.

Collected ticks were identified according to taxonomic keys of [11]. *B. decoloratus, A. variegatum* and *R. evertsi evertsi* ticks were incubated at 27°C and 80-90% relative humidity where they maintained until egg-laying and egg hatching was completed.

2.7. Chemical Acaricide and Distilled Water Preparation

According to the preliminary assessment made in the study area the most widely used chemical acaricide was Diazinol. Hence, commercially available preparations of Diazinol 60% EC produced by Kafr El Zayat pesticides and chemicals co. Egypt was purchased from locally existing veterinary pharmacies of the study district for the bioassay. Dilutions were made based on the manufacturer's recommendation and guidelines on leaflets with distilled water to prepare a concentration of 0.1% (1 ml Diazinol/1000ml water), 0.2% (2ml Diazinol/1000ml water), 0.4% (4ml Diazinol/1000ml water), 0.8% (8 ml Diazinol/1000ml water) and 1.6% (16ml Diazinol/1000 water) as a treatment while distilled water was used as a placebo.

2.8. Larval Packet Test (LPT)

The LPT, with few adjustments, was conducted as per [12]. The prepared acaricide and distilled water with a volume of 0.7 ml were applied to a 10 cm filter paper (Whatman No. 1, Whatman, Madstone, United Kingdom) and 20 larvae with the age of 15 to 20 days were exposed to the soaked (treated) filter papers and folded in half. The filter papers were then sealed with strong paperclips and incubated at 27 ± 1°C and 85 ± 5% relative humidity as designated by [13]. All tests were conducted in triplicate, using distilled water as a placebo. The paperclips were removed after 24 hours of incubation, placed on polystyrene block and larval mortality was appreciated. Only larvae capable of moving were considered as alive. All other larvae, including those that move appendages, were regarded as dead. The resulting mortality rate was then compared with negative controls to observe the statistical significance difference. Mortality was determined using the following formulas:

$$\text{Mortality (\%)} = \frac{\text{dead ticks larvae count}}{\text{total tick larvae}} * 100$$

$$\text{Pooled mean mortality (\%)} = \frac{\text{Mortality } i + \text{Mortality } ii \ldots + \text{Mortality } n}{N}$$

2.9. Data Organization and Statistical Analysis

The data from the questionnaire and experimental work were organized, entered and cleared using Microsoft Excel 2007 computer program and analyzed using SPSS V20 and Graphpad Prism version 7 Software. Chi-square ($\chi 2$) statistics was employed to check susceptibility differences between tick species exposed to different acaricide concentrations by setting 95% of confidence interval and $p<0.05$ were reflected to be statistically significant. The dose-response curve was sketched and the LC_{50} values of Diazinol was determined using GraphPad Software, 2016. To define the level of resistance conquered by the population after exposure to the insecticide or acaricide with bioassay, the impact of the accrued resistance genes on the success of the insecticide or acaricide would have to be compared with the effect of vulnerable genes on the effectiveness of the insecticide or acaricide, and not the field suggested concentration. The RF (resistance factor) of an arthropod pest population which designates the level of resistance would, therefore, be calculated using the following formula: [14-18]

$$\text{Resistant factor (RF)} = \frac{\text{LC50 value of field ticks}}{\text{LC50 value of susceptible ticks}}$$

3. Results

3.1. Questionnaire Survey Result

A total of 260 farmers, composed of 53 female (20.4%) and 207 male (79.6%), aged 19 to 78 years old, owning local (82%) and cross (18%) bred dairy animals were interviewed. About 94.6% of the farmers reported tick infestation on their animals. The seasonal occurrence of ticks infestation as reported by farmers was 45.1% in summer (Bega) (December, January February), 24% in autumn (Belg) (March, April, May) 12.6% in winter (Kiremet) (June, July, August), and 4.6% in spring (Tsedey) (September, October, November). Udder, tail and on scrotum, neck and leg, brisket, ear, anus, dewlap, (shoulder and arm), ventral side, back side, head and vulva), armpit, (groin and feet) were identified as the most frequently infested animal body parts with a magnitude of 21%, 12.4%, 10%, 9%, 8.8%, 6%, 5.8%, 5%, 2.3%, 1.9%, 1.5%, 1.2% and 0.4%, respectively. In addition, the most often external parasites observed and reported by the farmers were lice (63.9%), fleas (15%), mange mites (9%) and mixed infestation (12.1%).

Most of the farmers (69.4%) prepare (dilute) chemical acaricide to treat their animal by themselves based on professional advice (94.8%). About 74.6% the farmers treats only when there is occurrence of tick infestation and about 2.2% of the respondents told that the chemical is not satisfactory while 17%, 74.9% and 5.9% responded that the chemical is moderate, satisfactory and sometimes satisfactory respectively.

3.2. Experimental Results

Acaricide chemical and its concentration used are displayed in table 1 while the results of LPT are presented in tables 2 and 3 as well as in Figures 2, 3, 4, and 5. The pooled mean mortality rate was higher on all species of ticks at all concentration levels of both test and reference ticks (90% to 100%). At the concentration of 0.1%, 95% mortality was observed on both *A. variegatum and R. evertsi evertsi* while 90% mortality was recorded on *B. decoloratus*. The rest of the treatment concentrations produced 100% mortality on all test and reference ticks. The overall mean mortality of *A. variegatum, B. decoloratus*, *R. evertsi evertsi* and their references were 84.76%, 83.57%, 84.76% and 85.47% respectively. Significant ($P<0.05$) ($P=0.0001$) difference was observed statistically in the overall mean mortality among tick species and between their respective references. However, the mortality encountered at each concentration in all ticks species was statistically insignificant ($P>0.05$) ($P=0.1723$). Mortality of test ticks were increased as the concentration increased, while all reference ticks were killed at all concentrations (100%). Hundred percent mortality was also observed at a concentration of 0.2, 0.4, 0.8, and 1.6 on test ticks (Table 2).

The LC_{50} of Diazinol (%) for *A. variegatum*, *B. decoloratus*, *R. evertsi evertsi* and for their references were 0.03834, 0.0783, 0.0384 and 0.02926 respectively. *A. variegatum* and *R. evertsi evertsi* showed a resistance factor of 1.310 being susceptible to Diazinol while a resistance factor of 2.676 was encountered for *B. decoloratus* indicating a level 1 resistance. The dose-response data were analyzed by probit method and the log LC_{50} values calculated showed on (Figures 2, 3, 4 and 5).

Table 1. *Concentration level of Diazinol used for the acaricidal test.*

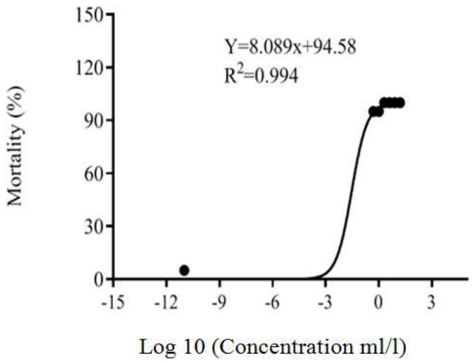
Concentration (ml of acaricide /1000ml of distilled water)	Percentage concentration V%= volume of solute ÷ volume of solution x 100	Equivalent concentration (ml of acaricide /100 ml of distilled water)
1 ml/1000ml	0.1%	0.01 ml/100ml
2 ml/1000ml	0.2%	0.02 ml/100ml
4 ml/1000ml	0.4%	0.04 ml/100ml
8 ml/1000ml	0.8%	0.08 ml/100ml
16 ml/1000ml	1.6%	0.16 ml/100ml

Table 2. *Pooled mean percentage mortality rate in test and reference ticks exposed to different concentration levels of Diazinol.*

Tick species	Mortality under Concentration (%)						Mean mortality	p-value
	Control	0.1%	0.2%	0.4%	0.8%	1.6%		
A. variegatum	3.33%	95%	100%	100%	100%	100%	84.76%	P<0.05 (0.0001)
B. decoloratus	3.33%	90%	100%	100%	100%	100%	83.57%	
R. evertsi evertsi	3.33%	95%	100%	100%	100%	100%	84.76%	
A. variegatum reference	3.33%	100%	100%	100%	100%	100%	85.47%	
B. decoloratus reference	3.33%	100%	100%	100%	100%	100%	85.47%	
R. evertsi evertsi reference	3.33%	100%	100%	100%	100%	100%	85.47%	
P-value	P>0.05 (P=0.173)							

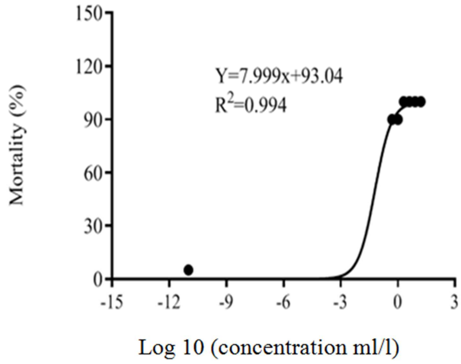
Table 3. *LC_{50} of Diazinol against test and reference ticks using the modified FAO larval packet test.*

Ticks	LC_{50} (%)	Standard error Lc_{50}	Degree of freedom	R^2
Amblyomma variegatum	0.03834	0.2624	5	0.9957
B. decoloratus	0.0783	0.1754	5	0.9929
R. evertsi evertsi	0.03834	0.2624	5	0.9957
Reference (All ticks)	0.02926	0.3484	5	0.9959



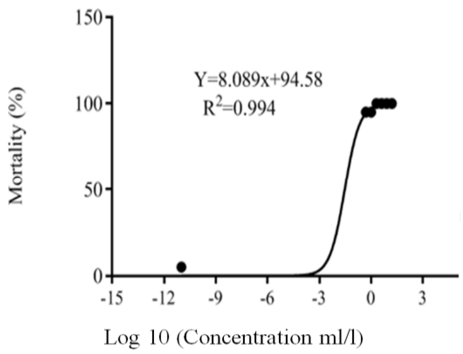
(Amblyomma variegatum)

Figure 2. *Dose response plot.*



(Boophylus decoloratus)

Figure 3. *Dose response plot.*



(Rhipicepalus evertsi evertsi)

Figure 4. *Dose response plot.*

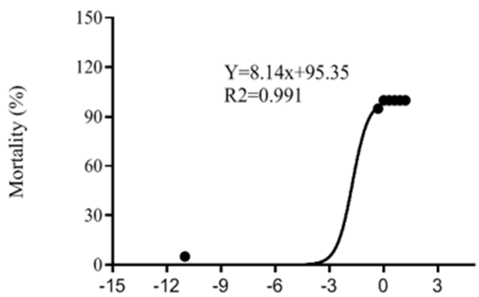


Figure 5. *Dose response plot.*

(Amblyomma variegatum, Boophylus decoloratus and Rhipicephalus evertsi evertsi) references.

4. Discussion

Resistance to pesticides is a genetically controlled phenomenon, supposed to progress largely as a consequence of selection of pre-adoptive mutants for detoxification, target site insensitivity, or other means of existence in the presence of insecticides. Acaricide resistance is nature's response to the extensive usage of continuing chemicals whose existence constitute a selective effect on genetically gifted survivors [19]. The purpose of the current study was to analyze Diazinol resistance status in field samples of *A. variegatum, B. decoloratus and R. evertsi evertsi* in Adea Berga district. The survey result indicated that tick infestation occurrence (94.6% prevalence) was throughout the year which is reliable with the study done by [20], though they reported a high tick prevalence rate was in the long rainy season (Kiremet) followed by a short rainy season (Belg). This variation might be due to the difference in altitude, temperature and humidity level of the study areas.

The current study confirmed that dilution and application of acaricides most of the time was accomplished by the farmers (69.4%) even though most of the farmers dilute and treat their animals based on professional advice (94.8%). This study indicated also about 74.6% of the farmers treat only when there is tick infestation incident which shows that the farmers do not have a prearranged tick prevention and control program [21]. The main source of acaricides were private veterinary pharmacies (93.9%) which is similar to the study done by [20], who reported private pharmacies (61.4%) and government veterinary clinics (15.7%) to be the core basis of acaricides. The majority (74.9%) of the farmers told the chemical is satisfactory while the rest 2.2%, 17%, and 5.9% of the respondents replied that the chemical is not satisfactory moderately satisfactory and sometimes satisfactory respectively. This indicates that even though farmers are not able to distinguish good quality acaricides from fakes, they can distinguish the good from bad by observing the reduction of tick infestation [22].

Information on the status and scale of acaricide resistance is of supreme importance in deciding the suitable tick and tick-borne disease control plan in different localities in Ethiopia [23]. Since the first report of the growth of resistance of *B. microplus* to arsenic in Australia in 1937 [24] and *B. decoloratus* in South Africa in 1939 [25], cases of numerous acaricide resistance by *Rhipicephalus*, *Boophilus microplus* have been reported also in Mexico [26]. The progressive evolution of resistance of ticks affecting cattle to almost all of the available acaricides has frustrated the exertions of cattle producers. For example, in-vitro bioassays were used to investigate 3939 tick populations of cattle; of these, the 57.6% that exhibited acaricide resistance development were largely limited to South America (Brazil), Central America (Mexico), and Asia (India). A total of 3391 of these tick populations were *R. (B.) microplus*, of which 2013 exhibited acaricide resistance development [27]. In the current study, we have evaluated the resistance level of *A. variegatum, B. decoloratus*, *R. evertsi evertsi* against

Diazinol using LPT method. Consequently, *B. decoloratus* demonstrated resistance (level 1) against the chemical. Quite a lot of reports also substantiate our findings. *B. decoloratus* was found to be resistant to Diazinon in West Shewa [10], to organochlorine compounds (Toxaphane) in Western Ethiopia [28] and to Toxaphane also in Western Ethiopia [29]. The resistance might be associated with its one-host behavior and possibly its prolonged exposure to treatments as indicated by [30]. Improper application techniques and failure to maintain adequate concentrations might also play a role for the observed resistance in the current study. Both *A. variegatum* and *R. evertsi evertsi* test ticks did not divulge any resistance to Diazinol which is similar to the finding of [10] who indicated also all strains of *A. variegatum* were found highly susceptible to tested acaricides including Diazinon.

5. Conclusion

Our study identified *B. decoloratus* as a resistant strain for Diazinol in the area while *A. variegatum* and *R. evertsi evertsi* were susceptible. Further investigation in a wider area of the district on the existence and seasonal dynamics of other tick species is mandatory. Future works should also look at the utilization of acaricides in a wider area of the district and their efficacy against the existing tick species. Furthermore, farmers' awareness on the utilization of acaricides should be enhanced through training.

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