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Invivo and Invitro Acaricide Efficacy Evaluation on Cattle Ticks in Selected Areas of Wolaita and Dawuro Zones, Ethiopia

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Abstract- This study was conducted from June, 2013 to May, 2014 in selected areas of Wolaita and Dawuro zones, with the core intentions of to assess the type and efficacy of the most frequently used acaricides in the areas, to identify the most prevalent cattle tick species and recommend the effective acaricide for cattle tick control. To achieve these objectives preliminary survey; invitro and invivo acaricide efficacy evaluation techniques have been conducted. The major tick species identified in order of their importance were *Rhip(Boophilus) decoloratus* (60.92%), *Amblyomma variegatum* (28.26%), *A. cohaerens* (7.82%) and *A. gemma* (3.0%). Diazinon 60%EC, Amitraz 12.5%, Ivermectin and Deltamethrin, according to their importance, was the acaricides frequently used in the areas. For the invitro technique, a total of 320 *Rhip(Booph) decoloratus* and 320 *A. variegatum* engorged adult female ticks were collected from each study sites and the standard modified adult immersion test (AIT) was employed for two successive round. On the other side of study, the efficacies of all the four acaricides at dose of concentration recommended by the manufacturer were assessed all the way through purposively selecting a total of 255 naturally tick infested cattle aged between 1 to 5 years.

Keywords: *acaricides, cattle, efficacy evaluation, invivo and invitro, ticks.*

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Abstract- This study was conducted from June, 2013 to May, 2014 in selected areas of Wolaita and Dawuro zones, with the core intentions of to assess the type and efficacy of the most frequently used acaricides in the areas, to identify the most prevalent cattle tick species and recommend the effective acaricide for cattle tick control. To achieve these objectives preliminary survey; invitro and invivo acaricide efficacy evaluation techniques have been conducted. The major tick species identified in order of their importance were *Rhip(Boophilus) decoloratus* (60.92%), *Amblyomma variegatum* (28.26%), *A. cohaerens* (7.82%) and *A. gemma* (3.0%). Diazinon 60%EC, Amitraz 12.5%, Ivermectin and Deltamethrin, according to their importance, was the acaricides frequently used in the areas. For the invitro technique, a total of 320 *Rhip(Booph) decoloratus* and 320 *A. variegatum* engorged adult female ticks were collected from each study sites and the standard modified adult immersion test (AIT) was employed for two successive round. On the other side of study, the efficacies of all the four acaricides at dose of concentration recommended by the manufacturer were assessed all the way through purposively selecting a total of 255 naturally tick infested cattle aged between 1 to 5 years. The invitro result indicates as most of *Rhip(Booph) decoloratus* dipped in Diazinon at 0.06% solution laid eggs and in this case about 59.92% control achieved. Conversely, only a few *Rhip(Booph) decoloratus* ticks dipped in Deltamethrin (%C=93.03%) and Amitraz 0.025% (%C=89.08%) had laid eggs. *Amblyomma variegatum* immersed in Amitraz 0.025% (%C=94.51%) and Deltamethrin (%C=94.06%) had laid small batches of eggs. In contrast, almost all of the ticks in the control group (water treated) have successfully laid eggs. The overall mean oviposition response inhibition of both Amitraz 0.025% and Deltamethrin solution were higher than Diazinon 0.06% and it have shown statistically significant ($P<0.05$) variation. Diazinon 0.06% has also shown significantly fewer efficacies (79.79%C) than all the three drugs (Amitraz 0.025%=99.89%C, Deltamethrin 1%=99.22%C and Ivermectin 1ml/50Kg=99.14%C) in removing the adult ticks at field level assessment. Therefore, it can be recommended that, for effective cattle tick control in the area, threshold tick control approaches using most effective acaricide Amitraz, Deltamethrin and Ivermectin when tick infestation exceeds an acceptable level.

Keywords: acaricides, cattle, efficacy evaluation, invivo and invitro, ticks.

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I. Background and Justification

Ethiopia is a country that stands first in Africa and 10th in the world in the livestock population. The productivity of these animals is affected by many factors, among which animal diseases, inadequate nutrition, poor management, poor genetic makeup and recurrent drought are major causes. Dawuro and Wolaita zones of SNNP Region are potential for the production of livestock especially for cattle, sheep, goats, equine and poultry, but various diseases and disease related factors are affecting the production and productivity of these animals (CSA, 2007). The five years period (2005 – 2010) studies on the distribution of diseases by Sodo Regional Veterinary Laboratory have indicated that among more than 40 diseases and disease related problems stated by the live stock owners from 8 Zones (including Wolaita and Dawuro zones) and two special woredas, tick infestation stands second major problem (83.9%) following Blackleg. Ticks are of importance to veterinary medicine because they can be an annoyance, cause harm due to their blood feeding and they can transmit many pathologic organisms (Urquhart, 1996).

Tick infestation and tick born disease (TBD) control is based mainly on the use of acaricides, since alternative non-chemical tick control methods such as: predators and parasites, pasture spelling, sterile male release, use of tick resistant cattle, vaccination with tick antigens are either at experimental stage or have been shown to be inadequate. Thus, the most widely used method for effective control of ticks is the direct application of acaricides to host animals. However, acaricides are expensive and can be detrimental to the environment: their use should be minimized and integrated with alternative approaches (Cunningham, 1981; Minjauw and de Castro, 1999).

Repeated use of acaricides besides being the environmental hazard, it is exposed to be resisted by tick species through time, and this forces frequent application at high concentrations which is more critical to the environment. Tick acaricide resistance is reported in various parts of the countries where tick and tick borne diseases are of major problem. Since tick infestation is one of the major reported problems in the area, repeated use of acaricides is the only option in

high tick seasons (Jobre *et al.,* 2001). Therefore, this study was designed to identify the major cattle tick species in the area and to assess the efficacy of most frequently used acaricides for the control of ticks in selected sites of Dawuro and Wolaita zones, Ethiopia.

II. Materials and Methods

a) Description of study area

Wolaita and Dawuro zones of the SNNPR, the study areas, were located in southwest part of Ethiopia. The two zones are separated by the gorge of Omo River. Wolaita zone is located 380km south west of Addis Ababa with altitude between 700 and 2950 meters. Rainfall is with an annual average of 1200-1300mm. Mean monthly temperatures vary 11^0C to 26^0C. On the other hand, Dawuro zone is located at 512km south west of Addis Ababa. Altitude of the zone is 501-3000 meters with mean monthly temperature ranges 15.1^0C to 27.5^0C and annual rainfall of 1201-1800mm. Both zones practice a crop-livestock mixed farming and keep combination of livestock species. Equally, the zones receives a bimodal rainfall in short (February to March) and long (May to September) seasons. Agriculture is the main stay of rural livelihoods in the districts (CSA, 2007).

b) Study design

An experimental randomized controlled trial was used to assess the effect of Diazinon 60%EC, Amitraz 12.5%, Deltamethrin and Ivermectin against tick species under in-vitro and in-vivo on cattle kept under extensive and intensive production system in selected areas of Wolaita and Dawuro zones, Ethiopia.

c) Sample size determination

The sample size was determined by using the formula described by Schulz and Grimes (2005) by assuming =0.05, power=0.90 (table value=10.51) and equal sample size were used in the two groups (i.e. all treatment group and control). The overall expected efficacy of each tested acaricide was considered to be 100% according to Thrusfield (2005).

$$N = \frac{\text{Power } [(R+1)-P^2(R^2+1)]}{P^2(1-R)^2}$$ Where,

N= the sample size in each of the group

P1= Event rate in the treatment group

P2= Event rate in the control group

R= Risk ratio (P1/P2)

Thus, for each trial, the total sample size was 75 cattle

d) The study protocol

Prior to trial initiation, 150 ticks were randomly collected from cattle and preserved in screw cap bottles using 80% ethanol, 5% glycerin and 15% distilled water. The aim of the tick collection exercise is to get an idea on the tick species involved. Accordingly, the ticks were identified using the taxonomic criteria described by Kaiser (1987).

e) In-vivo acaricide efficacy evaluation trial

i *Experimental protocol*

Selection of cattle for the purpose of this experiment is dependent on two factors:

i) Presence of tick burdens in the farm/herd and

ii) Cattle that were not receive any acaricide treatment at least within one month from the commencement of the trial.

At all the study sites, a total of 255 naturally tick infested cattle aged between 1 to 5 years (from the dairy farm/38, Wolaita zone/114 and Dawuro zone/103) were purposely selected and randomly divided into five groups of cattle, each group having 6 to 10 cattle (n=6-10). Each selected animal was subject to each tested acaricide treatment (Diazinon 60%EC, Amitraz 12.5%, Ivermectin injectable, Deltamethrin and control). Each experimental cattle was identified with a name given by the owner, color of the animal, sex and other special marks on the animal.

f) Acaricide application procedures

Each tested Diazinon and Amitraz, was mixed with water at working dilution recommended as per the prescription of the manufacturer. Both Deltamethrin pour on and Ivermectin injection was also used as dosage prescribed by the manufacturer. And so, all cattle in Group-1, Group-2 and Group-3 were thoroughly wetted with freshly prepared emulsified concentrate of each tested acaricide at a volume and concentration recommended by the manufacturer. Cattle in Group-4 were injected with recommended dose of Ivermectin. Treatments were done only once at trial initiation (Day 0) after the first count of ticks (pre-treatment count). No acaricide was applied on cattle in Group-5 and they served as controls.

g) Ticks count on cattle

Basically, ticks were counted on the visible anatomical sites of half body, on alternative sides, of each cattle at defined body zones; namely the ears, head, dewlap, back, abdomen, anus-vulva and tail. All tick counts were conducted by the same person as per the procedure described by Bianchi *et al.* (2003). Ticks stage were identified and counted in situ, but none of them be removed. Tick collection was made regularly at defined intervals and time. Accordingly, counting was done at Day-0 (at trial initiation day) and then at D-7, D-14 and D-21 (after trial initiation days) (Ali Mohammed and De Castro, 1993). The parallel tick count results on Group-5 cattle were used as an index in computing the percentage tick control achieved (Rinkanya, 1984). Thus, the efficacies of one acaricide alone was estimated by comparing the tick loads on animals at the

time of the treatment (pre-treatment count) with those obtained at D-7, D-14 and D-21 after treatment and is calculated using the following formula described by Drummond, *et al.* (1981):

$$\text{Percent control} = \frac{\text{MTC-MTT}}{\text{MTC}} \text{X100}$$

Where, MTT and MTC are mean tick counts in treated cattle (Group-1, Group-2, Group-3 and Group-4) and untreated cattle (Group-5), respectively.

h) In-vitro acaricide efficacy evaluation trial

i *Adult Immersion Test (AIT): Oviposition response*

The in-vitro tested acaricides includes Diazinon 60%EC, Amitraz 12.5% and Deltamethrine which are commonly used by the communities and available at the market. For the evaluation of oviposition response inhibition of each tested acaricide, a total of forty (n=40) engorged female tick of each species of uniform size were collected from cattle and each tick species randomly allocated into four groups: Group-1 (n = 10), Group-2 (n = 10) and Group-3 (n = 10) are ticks subjected to each tested acaricide treatment and Group-4 (n=10) are untreated, ticks serve as control. During the study period, two successive replicates of the above trails for each acaricide treatment and control group were done. Therefore, during the study period a total of eighty (n=80) engorged female tick of each species were collected from cattle.

The weight of engorged female tick in all four groups was recorded. Ticks in Group-1 (n = 10), Group-2 (n =10) and Group-3 (n =10) were immersed in each evaluated acaricides at concentration recommended at field level. While ticks those assigned in Group-4 were immersed in distilled water. After 10 minutes of immersion all ticks were cleaned and air-dried at room temperature for an hour, pasted onto double-sided adhesive tape on glass test panels with their ventral sides facing upwards keeping their capitula clear of the tape and then were incubated at 25^{0}C to 28^{0}C and 85-90 % R.H. for 7 days. The effect of each tested acaricides on reproductive capacity of each immersed engorged female tick species was also determined and then compared with the control groups. All groups were then tested (evaluated) using the egg laying test method (Drummond, *et al.*, 1973 and modified by FAO, 2004) which involves the comparison of the egg mass of each engorged female tick treated in each tested acaricides with the egg mass of untreated engorged female tick and finally estimate the percentage control achieved by each test acaricide using the following formula:

$$\text{Percent control} = \frac{\text{MEC} - \text{MET}}{\text{MEC}} \text{X 100}$$

Where, MEC and MET are mass of eggs laid by control ticks and treated ticks, respectively.

i) Data management and Statistical analysis

All the collected data were entered to Microsoft Excel 2007 spread sheet then transferred to SPSS-Version 17. Descriptive statistics like mean and standard deviation were compared. Independent sample t-test was used to compare the mean tick burden between treated and control group. All analysis was performed at 95% CI and 5% significance level. After treatment, acaricides activity were assessed using arithmetic mean tick count which was calculated for treated and control group and the percentage reduction in mean tick count in both AIT and in-vivo tick count was determined as follows:

$$\%\text{efficacy} = \frac{\text{C-T}}{\text{C}} \text{x100}$$

Where:

C= Mean number of ticks/animal in the control group

T=Mean number of ticks/animal in the treatment group

III. Results and Discussions

a) Tick identification

The major tick species identified were *Rhipicephalus (Boophilus) decoloratus, Amblyomma variegatum, A. cohaerens and A. gemma. Rhip (Booph) decoloratus* was found to be the most prevalent tick species in the study areas. The total numbers of animals examined, total adult tick collected and identified from the different study areas were shown below in table-1.

Table 1 : Total adult tick collected and identified from the different study areas

Study area	Total animals	Total ticks	Tick species identified			
			Rhip(Booph) decoloratus	*A.variegatum*	*A.cohaerens*	*A.gemma*
Sodo zuriya	24	196	117	58	16	5
Dawuro zone	33	143	98	39	4	2
Dairy farm	11	160	89	44	19	8
Overall	68	499	304 (60.92%)	141 (28.26%)	39 (7.82%)	15 (3.00%)

b) The overall effect of in-vivo tested acaricides

i *The overall effect of Diazinon 0.06%*

The overall mean post treatment tick count result of Diazinon at 0.06% concentration had different efficacy at the Dairy farm, Sodo zuriya and Dawuro zone at each D-7, D-14 and D-21 post treatment (table-2). The result has shown higher statistical significant variation ($P<0.05$) of overall adult ticks removing in Dairy farm (98.17%) and Dawuro zone (75.56%) than Sodo zuriya woreda (65.64%).

ii *The overall effect of Amitraz 0.025%*

The overall mean pre-treatment tick count (D-0) was 286, 506 and 725 ticks in Dairy farm, Sodo zuriya and Dawuro zone, respectively. Following treatment with Amitraz 0.025% has showed statistically significant variation ($P<0.05$) between the overall mean pre-treatment and post treatment tick count at all the three study sites. Amitraz 0.025% results in maximum of 100% (D-21) total mean tick count reduction in Sodo zuriya and Dairy farm (table-2).

iii *The overall effect of Deltamethrin pour-on*

At all the three study sites, treatment of animals with Deltamethrin solution has shown the highest overall mean adult tick killing rate at D-21 of post-treatment. Deltamethrin has shown statistically significant ($P>0.05$) efficacy variation in removing adult ticks between Sodo zuriya woreda and Dairy farm at D-7 of post-treatment, and it was lesser at Sodo zuriya woreda (85.06%) than Dairy farm (95.73%).

iv *The overall effect of Ivermectin subcutaneous injection*

A similar, very good, efficacy was registered in the Ivermectin treated group at D-7 post treatment at Dawuro zone and Dairy farm, which has been maintained also at D-21 post treatment.

Table 2 : Total tick counts on cattle treated with each four tested acaricide and the %C achieved

Study sites	Type of acaricides	Day	Day-0	Day-7	Day-14	Day-21	Overall
Dawuro zone	Amitraz 0.025%	Treatment group	704	3 (99.64%)	2 (99.77%)	3 (99.61%)	8 (99.67%)
	Diazinon 0.06%	Treatment group	675	108 (87.38%)	166 (80.61%)	189 (75.56%)	463 (81.18%)
	Deltamethrin 1%	Treatment group	1020	48 (94.22%)	14 (98.36%)	8 (98.97%)	70 (97.15%)
	Ivermectin	Treatment group	615	41 (95.07%)	9 (98.95%)	5 (99.35%)	55 (97.76%)
	Water	% control	725	831 (99.82%)	856	773	2460
Sodo zuriya	Amitraz 0.025%	Treatment group	837	1 (99.82%)	1 (99.82%)	0 (100%)	2 (99.88%)
	Diazinon 0.06%	Treatment group	587	114 (78.97%)	156 (74.00%)	201 (65.64%)	471 (72.73%)
	Deltamethrin 1%	Treatment group	692	81 (85.06%)	7 (98.83%)	5 (99.15%)	93 (94.62%)
	Ivermectin	Treatment group	562	157 (71.03%)	40 (93.33%)	16 (98.97%)	213 (87.67%)
	Water	% control	506	542	600	585	1727
Dairy farm	Amitraz 0.025%	Treatment group	286	6 (97.17%)	3 (98.56%)	0 (100%)	9 (98.60%)
	Diazinon 0.06%	Treatment group	194	14 (93.36%)	9 (95.67%)	4 (98.17%)	27 (95.77%)
	Deltamethrin 1%	Treatment group	209	9 (95.73%)	4 (98.08%)	1 (99.54%)	14 (97.81%)
	Ivermectin	Treatment group	154	12 (94.31%)	5 (97.60%)	2 (99.09%)	19 (97.02%)
	Water	% control	203	211	208	219	638

c) The overall effect of in-vitro tested acaricides

Table-3 summarizes the total tick counts on the treated and control groups, and percentage control achieved during the invitro trial. The result indicates as most of the engorged female *Rhip (Booph) decoloratus* dipped in Diazinon at concentration of 0.06% solution laid eggs and in this case about 59.92% control was achieved. Conversely, only a few female *Rhip (Booph) decoloratus* ticks dipped in Deltamethrin solution and engorged female *Amblyomma variegatum* immersed in Amitraz at concentration of 0.025% solution and Deltamethrin had laid small batches of eggs. On the other hand, almost all of the ticks in the control group (water treated) have successfully laid eggs. As shown on table-4, through the in-vitro efficacy evaluation test, both Amitraz and Deltamethrin showed higher statistically significant ($P<0.05$) oviposition response inhibition than Diazinon. The mean, minimum and maximum overall oviposition response inhibition of each tested acaricides was listed on table-5 below. Accordingly, the highest mean oviposition response inhibition was recorded by Deltamethrin (93.54%) followed by Amitraz (91.79%) and Diazinon (65.3%).

Table 3 : Mean oviposition response of adult *A. variegatum* and *Rhip(Booph)* decoloratus after immersion in tested acaricide at field recommended concentration and 7 day incubation

Trail	Tick species	Acaricides	N	Eng.wght (gm)	S	No.LE	Egg M (gm)	%C
Trail-I	*B. decoloratus*	Amitraz	30	8.01	1	1	0.03	96.91
		Diazinon	30	7.56	7	6	0.31	68.04
		Deltamethrin	30	7.77	1	1	0.04	95.87
		Control	30	8.04	30	25	0.97	
	A. variegatum	Amitraz	30	8.28	4	3	0.07	92.86
		Diazinon	30	7.75	10	8	0.32	67.35
		Deltamethrin	30	8.39	1	1	0.06	93.88
		Control	30	8.03	27	24	0.98	
Trail-II	*B. decoloratus*	Amitraz	30	7.89	3	3	0.21	81.25
		Diazinon	30	8.26	7	6	0.54	51.79
		Deltamethrine	30	7.20	3	2	0.11	90.19
		Control	30	7.76	27	22	1.12	
	A. variegatum	Amitraz	30	7.72	1	1	0.04	96.15
		Diazinon	30	7.57	6	5	0.27	74.04
		Deltamethrin	30	8.23	2	2	0.06	94.23
		Control	30	7.92	26	23	1.04	

Table 4 : Multiple comparisons-of Percent control of the acaricides

(I) Acaricide type	(J) Acaricide type	Mean difference	Std.Error	Sig.	95%CI	
Amitraz	Deltamethrin	-1.75	4.31	0.976	-14.54	11.04
	Diazinon	26.48	4.31	0.00*	13.7	39.28
Deltamethrin	Amitraz	1.75	4.31	0.976	-11.04	14.54
	Diazinon	28.24	4.31	0.00*	15.45	41.03
Diazinon	Amitraz	-26.49	4.31	0.00*	-39.28	-13.70
	Deltamethrin	-28.24	4.31	0.00*	-41.03	-15.45

Table 5 : Overall mean percent oviposition control of tested acaricides at field recommended concentration against adult female A. vareigatum and B. decoloratus

Acaricides	Min. Efficacy (%)	Max. Efficacy (%)	Mean efficacy (%±SD)
Amitraz 0.025%	78.38	100	91.79±7.25
Deltamethrin 1%	90.19	95.87	93.54±2.4
Diazinon 0.06%EC	61.11	97.06	65.3±9.5

IV. Conclusion and Recomendations

Amitraz 12.5% at field recommended concentration of 0.025% provides relatively a higher oviposition response inhibition of each *Rh. pulchellus* and *A. gemma* than Diazinon 60%EC at 0.06% concentration; but it isn't statistically significant variation. However, both acaricides showed relatively less effect against the oviposition of *Rh. pulchellus* than against oviposition of *A. gemma*. Regard less of the tick species, each evaluated acaricide had variable efficacy against oviposition responses of *A. gemma* and *Rh. pulchellus* with higher significant ($P<0.05$) percent oviposition control of Amitraz 0.025% than for Diazinon 0.06%. For Diazinon, but not for that of Amitraz, at field recommended concentration the mean oviposition %C is slightly below the International and National standards of most African countries ($\geq$85%C Vs 80%C). A long time usage of one acaricide type, abnormal concentration, usage of unknown acaricides type/source, and frequent or none-programmed use of acaricides are the common phenomenon of tick control methods in the area. Therefore, from the present study it was recommended that threshold tick control approaches using most effective acaricide when tick infestation exceeds an acceptable level in the area. Educating and/or awareness creation for farmers on the

ways of proper acaricide usage, application, dilution and systematic ways of substitution has also its own contribution. It would be valuable to conduct this in-vitro test using different tick species or other efficacy evaluation methods involving larval and nymphal stage. Further In-vivo efficacy trial (trial at field level) should be conducted to assess the residual effect of these acaricides. From government part attentions should be given: on strengthening veterinary service delivery, effective legislation of acaricide importation, marketing and monitoring in the area

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