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Prevalence of Bovine Ticks and Short Residual Efficacy of Acaricides in Arba Minch Areas South West, Ethiopia

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Abstract: Cross - sectional study was conducted in Arba Minch areas South West, Ethiopia to determine the prevalence of bovine ticks, species composition and assess the efficacy of commonly used acaricides (amitraz and diazinon) against cattle ticks. From 384 randomly selected cattle, 816 ticks were collected and identified into species. The overall prevalence of cattle tick was 84.9%. *Amblyomma variegatum* was the most common species and its prevalence accounted 71.4%. Laboratory susceptibility test was done by exposing adult ticks to the recommended dose of 20 ml both for amitraz (1:625) and diazinon (1:1000) concentration. Ticks were susceptible to amitraz and diazinon in laboratory test. In field efficacy test, both amitraz and diazinon resulted significant reduction in mean tick count compared to the control, although amitraz showed a relatively better efficacy and long residual effect. Though ticks were susceptible to both amitraz and diazinon, amitraz showed better performance in field efficacy test. Hence, the use of the two acaricides is still promising, but may require repeated application. Strategies involving early detection of resistance could be recommended for long term use of the existing tools.

Key words: Acaricide · Amitraz · Diazinon · Efficacy · Prevalence · Ticks

INTRODUCTION

Ticks are obligate hematophagous ecto-parasites and vectors of animals and human diseases [1]. Tick-borne pathogens affect 80% of the world cattle population and the estimated annual global loss due to ticks is between US$ 13.9 billion and US$ 18.7 billion [2]. They are the focus of the emerging one health approach because they transmit multiple disease causing pathogens of humans and animals [3]. Ticks and tick-borne diseases are also among the major parasitic diseases of animals in Ethiopia [4] and are responsible for blood loss, tick worry, downgrade hides, skins and inject toxins [5]. Ticks transmit protozoan (e.g. theileriosis and babesiosis), rickettsial (e.g. anaplasmoses and heart water or cowdriosis) and viral diseases affecting livestock, wild animals and humans [6].

In Ethiopia, a study shows that *Amblyomma*, *Boophilus*, *Haemaphysalis*, *Hyalomma* and *Rhipicephalus* reported to be predominant and economically important genus. *Amblyomma variegatum* (vector of *Cowdria ruminantum* and *Theileria mutans*) and *B. decoloratus* (vector of *Anaplasma marginale* and *Babesia bigemina*) are among the most important ticks species [7-9].

The most common method to control ticks is use of different types of chemical acaricides which are used in different method of applications such as dressing, spraying, systemic and dipping. The application of acaricides is still the most effective method of ticks' control. These chemicals are acaricides of different groups such as organochlorines, organophosphates, carbamates, amidines or synthetic pyrethroids. However, the excessive use of the chemicals, uncontrolled applications of acaricides may have accelerated the emergence of tick resistance to several active ingredients available [10].

The efficacy of chemical acaricides can be investigated in-vitro or in-vivo trials to choose the appropriate acaricides [11, 12]. The development and application of acaricides contribute to prevention of tick-borne diseases. Diazinon and amitraz are cheap chemicals widely used for tick control and other ecto-parasites in Ethiopia.

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Regardless of their application, the prevalence of cattle ticks remains high in most part of the country including the study areas [13]. Moreover, ticks can evolve acaricides resistance through years due to repeated exposure to chemicals [14]. The information on efficacy of diazinon and amitraz and their effective residual time is scarce. Ticks response to the diazinon and amitraz could be studied to see for alternative acaricides. In addition, understanding the prevalence and species composition of tick is important to design tick control approach. This study aimed to assess the prevalence and species composition of ticks and the diazinon and amitraz efficacy against cattle ticks in the Arba Minch areas, southern Ethiopia.

MATERIALS AND METHODS

Study Area: This study was conducted in the Arba Minch Zuria district of two villages; Chano Mille and Shelle Mella. Ticks species composition and their prevalence were studied in Chano Mille village, the village is located at 6°6.666' N and 37°35.775' E and at altitude of 1, 206 m above sea level (masl). The average temperatures were 17.9 and 30.2°C. Domestic animals are usually kept in compounds in open conditions, but a few households use separate roofed cow shelters. The cattle population of Chano Mille was 2858. Cattle ranching are also one of their main sources of income [15].

Acaricide efficacy test was conducted in the Shelle Mella village. The village was selected purposely because the number of ticks per cattle (on average 20 ticks as shown in the baseline survey) was high and is appropriate for efficacy trial. The altitude ranges from 1001-2500 masl. Its temperature range from 23-25.8°C and mean annual rainfall ranges from 800-1200mm. The village is close to the Lake Chamo. Cattle ranching are also one of their main sources of income. The cattle population of Shelle Mella was 5625 [15]. The distance between the two villages is 32 km, in between there is Arba Minch town, the capital city of Gamo zone.

Study Animal and Tick's Prevalence Study Design: A cross-sectional study was conducted to assess the prevalence of cattle ticks species in Chano Mille village. The entire body surface of the cattle was thoroughly examined to collect adult ticks [7]. All ticks were removed from the entire body of each cattle and persevered in 70% ethanol with respect to age and sex, the locality, owner's name, animal identification code, date and month of the collection. The attachment site and sex of ticks also recorded. The age of the cattle grouped into young (1 to 2 year), adult (3 to 7 years) and old (> 8 years) while body condition grouped into poor, medium and good [16]. Then, the ticks identified at genus and species level in National Animal Health Diagnostic and Investigation Center (NAHDIC) at Sebeta by stereomicroscope using the standard taxonomic identification key.

Sample Size Determination for Tick's Prevalence: The sample size was determined based on the expected prevalence of 50%; confidence level of 95% and 5% desired absolute precision. As result a total of 384 cattle were sampled from study village. To increase the precision of the study, 5% of the sample was added and then the total sample size became 404. The number of male and female was proportional in the sampled population (N = 185 male and N = 219 female), Thru field [17].

Sampling Technique for Tick Prevalence Study: The total number of households with cattle and number of cattle in each household was taken from Agricultural office of the Chano village. The first household was selected randomly by lottery method and every K^{th} household was included. Where K calculated by the formula:

$$K = \frac{N}{n}$$

where
K = The gap between every household
N = Total number of households in Chano Mille
n = The sample size calculated (404)

Only one cattle from each household was included randomly after clustering in sex, age and body condition to minimize the bias.

***In-vitro* Susceptibility Test:** *In vitro* test was conducted in the laboratory to assess the susceptibility status of cattle ticks to amitraz and diazinon. Adult ticks were collected in the field from different herd and then it placed in plastic flasks pre-labeled with time, date and place of collection. The collected ticks were transported to Arba Minch University Medical Entomology Laboratory for susceptibility test using modified adult immersion test (AIT) [18].

The recommended concentration of acaricides (1:1000 for diazinon, 1:625 for amitraz) was used for the test. For each acaricide, there were three replicates each containing 10 ticks. Ticks were immersed in 20 ml of water or into 20 ml of each acaricide in a 100 ml plastic container.

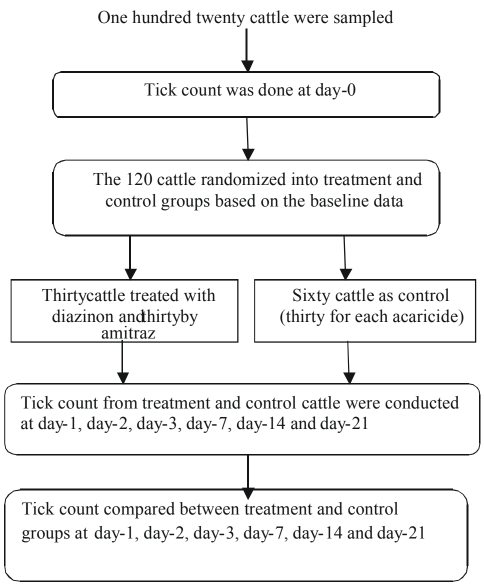

Fig. 1: Study design of acaricide efficacy test (September, 2016)

Ticks in all treatment and control groups' were transferred to Petri dish. Distilled water was used as a control. The number of ticks live or dead was counted after 24 h of exposure. The experiment was repeated three times for precision.

Acaricide Efficacy Study Design: A pilot trial was conducted on local breed cattle to evaluate the efficacy of acaricides diazinon and amitraz. The cattle were selected from the local breed in Shelle Mella village. Cattle that appear healthy, higher visible tick infestation and no history of ecto-parasites treatment at least four weeks prior to the start of the trial were included. Lactating animals were not included to reduce the risk of milk contamination. Those animals kept in houses (not moving for watering and feeding to the field) during the study period were not included to reduce the variation in exposure to environmental variables. Then, the baseline count of the number of ticks in all anatomical sites of the body of each cattle was done to randomize cattle into treatment and control groups.

A total sample size was 120 cattle. We randomized the cattle into four groups based on the baseline tick number. One group received diazinon (30 cattle), the second group received amitraz (30 cattle) and the other two groups (30 cattle for each) treated with water and used as control groups (Figure 1).

The acaricides were sprayed manually all over the animal body, but more emphasis was given on tick-infested areas. The acaricides' concentration was based on the manufacturer's recommendation for hand spray (1:1000 for diazinon, 1:625 for amitraz). The effect of the acaricides on ticks was observed at day-1, day-2, day-3, day-7, day-14 and day-21 of trial starting days. The efficacy of acaricide was estimated by comparing the tick number on treatment and control groups.

Sample Size Determination for Acaricide Efficacy Test: The sample size of the cattle involved in the study was calculated using the formula described by Kenneth and David [19] by assuming 95% confidence level (0.05), power = 0.90 (table value =10.51) and equal sample size in treatment and control groups. The overall expected efficacy of the tested acaricide considered 100% [20].

$$n = \frac{power[(R+1) - P_2(R^2+1)]}{P_2(1-R)^2}$$

where

n = the sample size in each of the group

P_1 = event rate in the treatment group

P_2 = event rate in the control group

R = risk ratio (P1/P2)

90% reduction was assumed: cut-off point according to FAO [18], then event in control group was 84.9% from our prevalence study. Hence, the minimum sample size was 11 cattle in each group. However, we included 30 cattle to increase the precision by minimize the variability due to extensive farming.

Ethical Considerations: Consent obtained from cattle owners and the livestock Office of the Woreda. Lactating animals were not included in the study to prevent milk contamination and the cattle used as control group during efficacy trial were treated by acaricide at the end of the study.

Data Analysis: We used SPSS software, version 20 for data analysis. The overall prevalence of tick was determined by dividing the number of positive cases by total number of cattle included in the study. Paired samples t-test was used to compare the efficacy of the acaricide at day-1, day-2, day-3, day-7, day-14 and day-21. The mean's ratio of ticks between treatment and control cattle was used to determine the efficacy of acaricide (percentage reduction) at different days after treatment. All statistical analyses considered significant at $P < 0.05$.

RESULTS

Prevalence of Tick Infestation and Species Composition: The overall prevalence of tick infestation in the study area was 84.9% (343 cattle were positive at least for one tick) (Table 1). Four tick genera (*Amblyomma, Boophilus, Hyalomma* and *Rhipicephalus*) were documented in the study area. There were nine species namely *Amblyomma variegatum*, *A. cohaerens, A. lepidum*, *H. marginatum rufipes*, *B. decoloratus*, *Rhipicephalus pravus*, *R. praetexatus*, *R. pulchelus* and *R. evertsi-evertsi*. *Amblyomma variegatum* was the most prevalent species with the prevalence of 71.4% (n = 245), followed by *A. cohaerens* 6.7 % (n = 23).

Of the total of 816 ticks collected, 536 was *A. variegatum,* the dominant species, accounted for 65.7%, followed by *A. cohaerens* 10.3% (n = 84). *Boophilus decoloratus (*n = 32) and *A. lepidum* (n =31) accounted for 3.9% each. The rest accounted for 16.2%.

Susceptibility Tests: Table 2 shows the percentage mortality of cattle ticks due to amitraz and diazinon acaricides after 24 hrs. *Amblyomma variegatum*, *A. cohaerens, B. decoloratus* and *Rhipicephalus* species were susceptible to diazinon and amitraz with 100% mortality. The control mortality was observed in *A. cohaerens* only with the average mortality of 5.6% in the control groups.

Field Efficacy of Acaricide Diazinon: There was no significant variation in baseline number of mean number of ticks at day-0 between control and intervention groups (Table 3). The variation was significant following the treatment with acaricide diazinon starting from day-1 to- day-14 but its efficacy reduced after day-7. The percentage reduction was highest (83.3%) at day-3 post treatment compared to control, but it was below the FAO cut off point, which is more than 90% (Table 3).

Table 1: Prevalence of tick species on cattle (n = 404) in Chano village in the Arba Minch area, southwestern Ethiopia (May-June, 2016)

Tick species	No. of positive cattle	Prevalence % (95% CI)
A. variegatum	245	71.4 (66.3-76.2)
A. cohaerens	23	6.7 (4.3- 9.9)
B. decoloratus	16	4.6 (2.7- 7.5)
A. lepidum	11	3.2 (1.6-5.7)
R. evertsi evertsi	8	2.3 (1.0-4.5)
R. pravus	2	0.6 (0.07-2.1)
R. pulchelus	2	0.6 (0.07-2.1)
***H. marginatum rufipes*	1	0.3 (0.007-1.6)
*Mixed infestation	35	10.2 (7.2-13.9)
Overall	343/404	84.9 (81-88)

*No. of cattle with mixed infestation, ** no mixed infestation

Table 2: The in vitro susceptibility test of diazinon and amitraz against cattle ticks from Shelle Mella village in the Arba Minch area district, southwest Ethiopia (September, 2016)

	Diazinon				Amitraz			
Tick species	≠ ticks in treatment	≠ dead after 24 h (%)	≠ ticks in control	≠ dead after 24 h (%)	≠ ticks in treatment	≠ dead after 24 h (%)	≠ ticks in control	≠ dead after 24 h (%)
A. variegatum	62	62(100)	113	0	66	66(100)	76	0
A. cohaerens	109	109(100)	44	10 (22.7)	110	110(100)	74	8 (10.8)
B. decoloratus	35	35(100)	30	0	14	14(100)	24	0
Rhipicephalus spp.	46	46(100)	1	0	38	38(100)	10	0
Total	242	252(100)	178	10 (5.6)	228	228(100)	184	8 (4.3)

Table 3: The efficacy of diazinon on field trial in Shelle Mella village in the Arba Minch area district, southwest Ethiopia (September, 2016)

Days (trials)	Mean ± SE	T	DF	Sig (two tailed)	% reduction
Baseline (Day-0) treatment	26.8± 2.9	0.16	29	0.87	--
Baseline (day_0) control	26.0±3.0				
Day-1 treatment	7.7 ±0.8	-5.2	29	0.001	70
Day-1 control	25.8±3.0				
Day-2 treatment	4.5±0.7	-6.9	29	0.001	80.8
Day-2 control	27.6±3.2				
Day-3 treatment	3.0±0.56	-7.9	29	0.001	83.3
Day-3 control	26.3±2.8				
Day-7 treatment	20.1±2.3	-2.0	29	0.05	32.7
Day-7 control	29.9±3.9				
Day-14 treatment	24.0±2.4	-2.3	29	0.03	28.4
Day-14 control	33.7±3.8				
Day-21 treatment	31.1±3.4	-0.9	29	0.38	12.3c
Day-21 control	35.4±3.8				

SE = Standard error; DF = degree of freedom

Table 4: The efficacy of amitraz on field trial in Shelle Mella village in the Arba Minch area district, southwest Ethiopia (September, 2016)

Days (trials)	Mean ± SE	T	DF	Sig (two tailed)	% Reduction
Baseline (day-0) treatment	20.9±9.8	-0.36	29	0.74	
Baseline (day-0) control	21.8±12.3				
Day-1 treatment	1.3±1.5	-0.36	29	0.001	93.5
Day-1 control	21.5±13.9				
Day-2 treatment	0.6±1.4	-7.88	29	0.001	96.4
Day-2 control	16.7±10.9				
Day-3 treatment	0.3±0.8	-7.66	29	0.001	96.6
Day-3 control	8.8±8.8				
Day-7 treatment	7.6±9.1	3.15	29	0.004	57.1
Day-7 control	17.7±13.2				
Day-14 treatment	12.7±11.9	-2.9	29	0.007	45.8
Day-14 control	23.4±15.4				
Day-21 treatment	13.6±11.4	-2.69	29	0.012	42.8
Day-21 control	23.7± 15.8				

SE = standard error; DF = degree of freedom

Field Efficacy of Acaricide Amitraz: The control and intervention groups had no significant difference at the baseline (at day-0) (Table 4). The reduction in tick number was significant from day-1 to- day-21 post treatment. The percentage reduction was substantially high until day-3 of post treatment. The efficacy was greater than 90% (the cut point of FAO) in the first three days of the trial. Amitraz removed 96.6% ticks from the body of infested cattle at day-3 post treatment, whereas the diazinon removed only 83.3% of cattle ticks in the same time.

DISCUSSION

The present study demonstrated high prevalence (84.9%) of cattle tick in the study area, implies high ticks infestation. The four genera and nine species of ticks namely *A. variegatum, A. cohaerens, A. lepidum, H. marginatum rufipes, B. decoloratus, R. pravus, R. praetexatus, R. pulchelus* and *R. evertsi-evertsi* were documented. *Amblyomma variegatum* was the most prevalent tick species. The result of field trial revealed that both diazinon and amitraz are effective against cattle ticks with short residual efficacy. There was a significant reduction until day-14 for diazinon and day-21 for amitraz. The acaricidal efficacy of amitraz was superior over diazinon as it showed maximum percentage reduction and had long residual efficacy.

The present study revealed that the overall prevalence of cattle tick in the Chano Mille village was 84.9%. This implies high prevalence of cattle ticks in the study area. The high prevalence of cattle ticks in turn may imply the extent of economic loss due to skin damage, weight loss and diseases. Similar findings were reported from Bedele district, Oromia region [21] and western Amhara regional state [22]. Some studies documented relatively low prevalence of cattle ticks [23-25]. The geographical differences, climatic conditions and cattle management practices might justify this variation [4]. Further detailed study could be done to assess the seasonal dynamics.

In present study, nine species of ticks were documented in four genera: *Amblyomma*, *Boophilus*, *Hyalomma* and *Rhipicephalus*. *Amblyomma variegatum* was the dominant cattle tick species. This was in line with the report that documented the dominance of *Amblyomma* and *Boophilus* cattle infesting ticks in Ethiopia [26]. Several researches conducted in different parts of the country indicated the dominance and wide distribution of *A.variegatum* and it is called all weather ticks [4, 27]. *Amblyomma variegatum* causes cowdriosis or heart water in cattle and it has great impact on damage to hides, skins because of their long mouth structure if infestation is high, downgrade the skin value in the world market and it decreases the nation's economy [28]. The presence of *A. variegatum* in a very high number was probably due to geographic location of the area and due to its being relatively active throughout the year.

The present study of susceptible test revealed that *Amblyomma*, *Hyalomma*, *Boophilus* and *Rhipicephalus* species were susceptible to 0.06% diazinon and 025% amitraz with 100% mortality within 24 hrs post exposure. In most of the studies, amitraz revealed high degree of mortality rate in line with the current study results by Mekonnen *et al.* [29] and a closely comparable finding with by Mekonnen *et al.* [30].

The efficacy of diazinon at day-3 was 83.3 %, whereas it was 96.6% for amitraz implies that amitraz at recommended concentration provided superior efficiency at the field trial on cattle in extensive management system. Also, amitraz showed superiority over diazinon in dairy farm in cattle [31]. Both acaricides showed lower efficacy in current extensive cattle management system than study done in intensive farm. However, this finding was comparable with the finding from Borena in southern rangeland of Ethiopia [31]. Moreover, internationally and nationally recommended cut-off of point for efficacy of acaricides is > 90% [18], but the efficacy of diazinon was lower than the recommended cut-off point. The efficacy of amitraz was higher than the cut-off point in the first three trial days but its efficacy declined shortly. Hence, on field trial, amitraz was superior than diazinon this is may be due to the long time usage of diazinon in the area.

CONCLUSION

Ticks are highly prevalent on cattle, which was 84.9 %. Four tick genera and nine species namely *A. variegatum, A. cohaerens A. lepidum, H. marginatum rufipes, B. decoloratus, R. pravus, R. praetexatus, R. pulchelus and R. evertsi-evertsi* were identified. *A. variegatum* was the most prevalent tick species with the prevalence of 71.4%, followed by *A. cohaerens* 6.7%. *Amblyomma* variegatum, *A. cohaerens, B. decoloratus* and *Rhipicephalus* spp. were susceptible to diazinon and amitraz with 100% mortality in the laboratory test. In the field efficacy trial, amitraz reduced the number of ticks significantly till day 21, whereas the reduction was significant due to diazinon only till day-7. The acaricidal efficacy of amitraz was superior over diazinon as it showed maximum percentage reduction and long residual efficacy.

Based on the finding of the present study, the following recommendation was forwarded; the acaricidal efficacy of amitraz was superior over diazinon, hence the use of amitraz may be considered for maximum tick's reduction.

A longitudinal study should be done to see the seasonal variation in species composition and identify where the ticks population is more abundant for effective intervention. Integrated control of tick infestation should be in place to tackle ticks infestation and improve the productivity of animals.

Conflict of Interest: The authors declare that there is not any conflict of interest with other concerned body in order to writing this article.

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