In vitro and in vivo acaricidal efficacy study of amitraz and diazinon against some tick species infesting Camelus dromedarius around Jigjiga, Eastern Ethiopia

Teka Feyera, Petros Admasu*, Befekadu Urga Wakayo and Mulisa Megersa

College of Veterinary Medicine, Jigjiga University, P.O.Box 1020, Jigjiga, Ethiopia.

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Tick infestation is considered to be a major concern as ticks cause widespread distress, act as vectors of disease and affect the economic conditions of livestock-rearing. Although the use of chemicals is still the most effective method of tick control, uncontrolled applications may have accelerated the emergence of tick resistance to several active ingredients available. The present work estimated the efficacy of two commonly used acaricides (amitraz and diazinon) against Rhipicephalus pulchellus and Hyalomma dromedarii collected from Camelus dromedarius by using in vitro and in vivo field trial methods. The in vitro test employed a preliminary immersion technique and the field trial involved a manual application of the acaricides on camels predominantly infested with both tick species under field condition. Three groups of camels (I, II and III) containing six head of animals each with a mean number of >20 tick counts received diazinon, amitraz and distilled water, respectively. The in vitro assay showed no statistically significant tickicidal difference (p>0.05) between these compounds although amitraz proved a relatively better efficacy. For both acaricides, doubled concentration was more effective in tick killing. The field trial evidenced that both drugs caused a significant reduction (p<0.05) in mean tick count when compared to the negative control. However, amitraz showed a significant superiority (p<0.05) as compared to diazinon (85.2%) eliminating almost all ticks from the body of infested camels 72 h post treatment with 99.1% efficacy. Whereas, the maximum antiparasitic efficacy brought about by diazinon was 85.2% after 72 h of application. In conclusion, comparing the efficacy of the two acaricides from these preliminary tests, amitraz is the preferable one. Furthermore, right application and choice of acaricides needs to be pursued in order to avoid any resistance against ticks in camels.

Key words: Amitraz, camel, diazinon, efficacy, Rhipicephalus pulchellus, Hyalomma dromedarii.

INTRODUCTION

A wide range of internal and external parasitic diseases are found in domestic animals. Among external parasites, ticks are undoubtedly the most economically important ectoparasites of livestock on global scale (Zenebe, 2005).
Ticks are destructive blood sucking ecto-parasites, found in most if not all the countries of the world, but are of greater economic importance in the tropical and sub-tropical zones (Sieg mund, 1979). Ticks cause widespread distress and morbidity and act as vectors of disease and affect the economic conditions of camel-rearing. Apart from transmitting diseases: they also reduce milk, meat production and increase susceptibility to other diseases. It may also lead to skin rejection at tannery factories (Walker et al., 2003).

Among 60 tick species found infesting both domestic and wild animal of Ethiopia, 30 species have been widespread and are important parasites of livestock (Solomon et al., 2004), and causes significant economic losses to the livestock industry. The most common tick affecting camel belongs to the genera: Ambylomma, Hyalomma, Dermacentor and Rhipicephalus (all hard ticks) or family Ixodidae (Kohler-Rollefson et al., 2001). The main effect of tick infestation in one humped camel is mild to severe anemia and loss of appetite, leading to a reduction in growth rate and decreased productivity. Tick infestation also results in increased calf mortality (Nelson et al., 1997).

The application of chemicals is still the most effective method of ticks control. However, uncontrolled applications of commercial acaricides may have accelerated the emergence of tick resistance to several active ingredients available. Since acaricide introduction in Africa around 1890, tick treatment relying on different application methods have been the main method of tick control in Africa, leading to numerous problems; environmental pollution, development of resistant tick strains and escalating costs (Brito et al., 2011). Likewise, in Ethiopia, over the past decades ticks are mainly controlled by using variety of acaricides; including organochlorines, organophosphates, carbamates, amidines or synthetic pyrethroids (Sil eshi, 2001; Yilma et al., 2001). However, with the most widespread, under or over concentration and frequent use of organochlorines and organophosphates compounds, ticks are likely to develop resistance in Ethiopia (Yilma et al., 2001; Sileshi et al., 2004). Therefore, continuous studies on dynamics of tick population (Alann, 2011) with the efficacy status of acaricides against the most abundant and important tick in particular area are necessary to carry out efficient tick control and/or tick burden reduction (Solomon and Kaaya, 2001).

In Eastern Ethiopia, high prevalence of ectoparasites especially ticks in camels have been reported (Bekele et al., 2012: Dinka et al., 2010; Zeleke and Bekele, 2004). However, due attention has not been given to the control of ectoparasites in camel. Information on the status and magnitude of ectoparasiticidal efficacy is no doubt of paramount importance in deciding the appropriate tick control strategy in camels in the specific area.

Therefore, the present study aimed at assessing the efficacy of two commonly used ectoparasiticidal agents, amitraz and diazinon, against the most abundant and important ticks infesting camels under in vitro and field condition.

**METHODOLOGY**

This preliminary investigation employed an experimental study design; laboratory based in vitro test and field efficacy trial, on two commonly utilized acaricides in and around Jigjiga, Somali Regional State of Ethiopia. The choice of these acaricides was based on their commercial availability and patronage by livestock keepers and veterinary clinics in the area.

**Tick Collection and Identification**

The entire body surfaces of the animals were examined thoroughly and adult ticks were collected from one side of the animal body. Ticks were collected from neck/dewlap, udder/scrotum, perineum/anus and legs/belly in combination. The bottles were labeled by considering the predilection sites and sampled animals. All collected ticks were examined under stereomicroscope and identified to the species level using the taxonomic key described by Kaiser (1987).

**In vitro acaricidal efficacy study**

The in vitro acaricidal efficacy study was conducted on two dominant tick species infesting camels in the area. Accordingly, unattached adult Rhipicephalus (R.) pulchellus and Hyalomma (H.) dromedarii were collected during field work and exposed to diazinon (Kat Relzayat Pesticides and Chemicals Co. Ltd, Egypt) and amitraz (Qiankuma Veterinary Pharmaceutical., CO.Ltd., China) according to Holdsworth et al. (2006) immersion technique. The recommended (1:1000 for diazinon, 1:625 for amitraz), double (2:1000 for diazinon, 2:625 for amitraz) and half doses were prepared. 1 ml of each liquor was added on petri dish with a filter paper fit at its bottom. Then, the acaricide was evenly distributed and 10 ticks of equal size were placed on each petri dish and it was closed. This was done separately for each species of tick. Distilled water was used as a control.

The number of ticks alive or dead was counted after 24 h of exposure. The experiment was repeated three times for precision and mean value was taken for the analysis.

**In vivo acaricidal efficacy study**

The field efficacy trial was conducted on camels naturally and predominantly infested with R. pulchellus and H. dromedarii ticks under field condition. Other tick species identified with minor number include H. truncatum, Amblyomma gemma, Amblyomma variegatum and Boophilus (Rhipicephalus) decoloratus. A total of 18 camels with a mean number of >20 tick counts on different body sites were admitted for the trial. These animals were randomly grouped in to three containing six head of camels each. The first group was treated with diazinon, the second group was treated with amitraz and the third group was left untreated as negative control. The acaricides were sprayed manually with more emphasis on tick infested regions of the body. The acaricides’ concentration was based on the manufacturer’s recommendation for hand spray (1:1000 for diazinon, 1:625 for amitraz). The tickicidal effects of the acaricides were observed at 24, 48 and 72 h post exposure. For both the in vitro and in vivo experiments, antiparasitic efficacy of each treatment was calculated using the following equation (Wang et al., 2009):

\[ AE = \frac{B - T}{B} \]
Table 1. *In vitro* ticks killing effect of diazinon and amitraz at double, recommended and half doses 24 hours post exposure.

<table>
<thead>
<tr>
<th>Treated tick</th>
<th>Treatment</th>
<th>NE</th>
<th>MNS</th>
<th>MND</th>
<th>AE (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>R. pulchellus</td>
<td>Amitraz RD</td>
<td>10</td>
<td>1.00±0.00</td>
<td>9.00±0.00</td>
<td>89.3</td>
</tr>
<tr>
<td></td>
<td>Amitraz DRD</td>
<td>10</td>
<td>0.00±0.00</td>
<td>10.00±0.00</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Amitraz HRD</td>
<td>10</td>
<td>3.33±0.58</td>
<td>6.67±0.00</td>
<td>64.3</td>
</tr>
<tr>
<td></td>
<td>Diazinon RD</td>
<td>10</td>
<td>1.67±0.58</td>
<td>8.33±0.58</td>
<td>82.1</td>
</tr>
<tr>
<td></td>
<td>Diazinon DRD</td>
<td>10</td>
<td>0.67±0.58</td>
<td>9.33±0.58</td>
<td>92.8</td>
</tr>
<tr>
<td></td>
<td>Diazinon HRD</td>
<td>10</td>
<td>6.00±0.00</td>
<td>6.00±0.00</td>
<td>35.7</td>
</tr>
<tr>
<td></td>
<td>Distilled water</td>
<td>10</td>
<td>9.33±0.58</td>
<td>0.67±0.58</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Amitraz RD</td>
<td>10</td>
<td>0.33±0.58</td>
<td>9.67±0.58</td>
<td>96.3</td>
</tr>
<tr>
<td></td>
<td>Amitraz DRD</td>
<td>10</td>
<td>0.00±0.00</td>
<td>10.00±0.00</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Amitraz HRD</td>
<td>10</td>
<td>3.00±1.00</td>
<td>7.00±1.00</td>
<td>66.7</td>
</tr>
<tr>
<td></td>
<td>Diazinon RD</td>
<td>10</td>
<td>0.67±0.58</td>
<td>9.33±0.58</td>
<td>92.5</td>
</tr>
<tr>
<td></td>
<td>Diazinon DRD</td>
<td>10</td>
<td>0.00±0.00</td>
<td>10.00±0.00</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Diazinon HRD</td>
<td>10</td>
<td>2.67±0.58</td>
<td>7.33±0.58</td>
<td>70</td>
</tr>
<tr>
<td></td>
<td>Distilled water</td>
<td>10</td>
<td>9.00±0.00</td>
<td>1.00±0.00</td>
<td>0</td>
</tr>
</tbody>
</table>

Values are mean ± SD; RD= recommended dose; DRD= Double recommended dose; HRD= Half recommended dose; NE= Number of ticks exposed; MNS=Mean number of ticks survived; MND= Mean number of ticks died; AE= Antiparasitic Efficacy.

Where AE is the antiparasitic efficacy, B is the mean number of surviving ticks in the control, and T is the mean number of surviving ticks in treatment.

### Statistical analysis

Data were organized, edited and analyzed using statistical package for social sciences (SPSS) Version 20. Results generated from the investigation were expressed using descriptive statistics (mean ± standard error of mean, percentage and graphs). One way analysis of variance (ANOVA), and the student t test was employed for inter-group and intra-group difference analysis. Results were deemed statistically significant if p≤0.05 at 95% confidence intervals.

### RESULTS

#### In vitro acaricidal efficacy

Analysis result of this preliminary test indicated no significant difference (p>0.05) between the two acaricides on the killing effect at any of the three different concentrations tested against both species of ticks (Table 1). Although not statistically significant, amitraz seemed to be superior to diazinon as measured by antiparasitic efficacy (%) estimation against *R. pulchellus*. However, both compounds appeared to have a comparable and relatively better efficacy (>90% at recommended dose) against *H. dromedarii*. Both compounds produced maximum efficacy only at their double recommended dose. The mean number of ticks died 24 h post exposure to distilled water was not more than one (Figures 1 and 2).

### DISCUSSION

In Ethiopia, amitraz and diazinon are one of the most commonly applied chemicals for tick control and treatment in different species of domestic animals. Attempts have also been made by different authors to assess the susceptibility of ticks to these two chemicals. There is a trend of variability in the level of efficacy in most of such studies (Sileshi 2001; Sileshi et al., 2002; Eshetu et al., 2013).

In the present preliminary study, the *in vitro* readings demonstrated that amitraz appeared show relatively better activity although it did not produce 100% tickicidal effect at its recommended concentration. The estimated antiparasitic efficacy for amitraz and diazinon was 89.3 and 82.1% against *R. pulchellus*, and 96.3 and 92.5% against *H. dromedarii*.
Table 2. Cumulative density of adult stages of ticks with efficacy % on the different observed regions of camels’ body before and after treatment.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean number of ticks (mean±SEM)/AE (%)</th>
<th>Pre-treatment</th>
<th>Post-treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>24 h</td>
<td>48 h</td>
</tr>
<tr>
<td>Diazinon</td>
<td>21.83±2.21</td>
<td>11.83±2.09a</td>
<td>6.17±1.87a</td>
</tr>
<tr>
<td>Amitraz</td>
<td>23.50±2.58</td>
<td>3.17±0.95ab</td>
<td>0.67±0.49ab</td>
</tr>
<tr>
<td>Negative control</td>
<td>20.67±1.38</td>
<td>20.33±1.33</td>
<td>19.67±1.38</td>
</tr>
</tbody>
</table>

Values are mean ± SEM; n = 6; SEM= standard error of mean; AE= Antiparasitic Efficacy; All superscripts indicate significance at p < 0.05 (a compared to infested untreated; b compared to diazinon group).

Figure 1. *In vitro* killing effect of diazinon and amitraz at double, recommended and half concentrations against *R. pulchillus*. RD= recommended dose; DRD= Double recommended dose; HRD= Half recommended dose.

Figure 2. *In vitro* killing effect of diazinon and amitraz at double, recommended and half concentrations against *H. dromedarii*. RD= recommended dose; DRD= Double recommended dose; HRD= Half recommended dose.

against *H. dromedarii* respectively. Diazinon was not able to eliminate all *R. pulchellus* ticks even at double concentration of its normal dose. In most of the previously conducted *in vitro* studies in Ethiopia and other
countries, amitraz revealed high degree of tickicidal efficacy which agreed with the present finding (Sileshi, 2001; Sileshi et al., 2002). Eshetu et al. (2013), compared the efficacy of amitraz and diazinon on R. pulchellus and other ticks and found that amitraz at recommended concentration provides better efficient oviposition inhibition than diazinon which agrees with the finding of this study. Sileshi et al. (2003) in South Africa observed relatively higher level of resistance to diazinon than amitraz. On the other hand, Melaku (2013) reported absence of significant difference in the in vitro efficacy of amitraz and diazinon but better in vivo efficacy of amitraz against Amblyomma ticks in Gondar.

Under in vitro condition, R. pulchellus was found to be relatively less susceptible to both compounds as compared to H. dromedarii. It can be speculated that R. pulchellus is more likely to develop acarical resistance because it infests ranges of domestic animals which might increase its exposure to repeated application of ectoparasiticidal agents in the field. However, both compounds seemed to have a comparable and relatively better in vitro efficacy (>90% at recommended dose) against H. dromedarii. Double concentration of both chemicals completely eliminated H. dromedarii ticks 24 h post exposure. It has been reported that camels are the preferred hosts of H. dromedarii (Walker et al., 2003). This study suggest that this limited host preference might have favored this tick species to have less prior exposure to acaricides such as amitraz and diazinon guarding it from the high likely hood of developing resistance as against R. pulchellus.

In vivo readings indicated a significant superiority (p<0.05) of amitraz in relation to reducing mean tick count as compared to diazinon during the three days observation period. It was able to eliminate almost all ticks from the body of infested camels 72 h post treatment with 99.1% efficacy. However, diazinon failed to eliminate all ticks from the body of animals although it caused significant reduction as compared to negative control. The presence of engorged ticks in diazinon treated camels can be a clue that it failed to inhibit growth and reproductive capacity of the ticks. This may be related to the decrease in the susceptibility of the tick to diazinon. This finding is in line with report of Turkson and Botchey (1999) who reported that field strain of some tick species is resistant to organophosphates like diazinon. Emergence of resistant tick against organophosphate acaricides was also stated by Tessema and Gashaw (2010). Melaku (2013) also reported compromised efficacy of diazinon against Amblyomma ticks in vivo. Similarly, Kumar et al. (2011) noted that the continuous use of diazinon results in the resistance of Rhipicephalus (Boophilus) microplus to diazinon in 20 locations situated at various regions of India. The frequency of application of acaricides, dilution rate, storage as well as the quality of the product will affect the effectiveness of an acaricide (Turkson and Botchey, 1999). The use of an acaricide at incorrect concentration has been reported as one of the prime factors which affect the efficacy of an acaricide and causes of tick control failure (Natala et al., 2005; Kirby, 2010; Alanr, 2011). There was also observation in the area that pastoralists apply these chemicals to their animals by themselves and believe that increasing the concentration of acaricides during application would benefit tick eradication. This type of increased acaricide concentration can lead to a higher selection pressure for tick resistance (Brito et al., 2011; Pegram et al., 2000).

The efficacy variation between the two presently tested acaricides might be associated with the high sterilization effect of amitraz compared to diazinon when applied at field recommended concentration (Sileshi et al., 2003). Amitraz is an important acaricide because it does have some valuable properties for tick control. Amitraz has been shown to be an excellent detaching agent, inducing the rapid detachment of live ticks from infested animals (Mekonnen, 2001; Natala et al. 2005). However, some studies have shown that amitraz can take several days to kill ticks and that some surviving ticks can complete engorgement and lay viable eggs. It is for these reasons that amitraz is an acaricide useful for tick control but not for tick eradication (Burridge et al., 2003). However, still amitraz is preferable than diazinon in inhibiting oviposition (Eshetu et al., 2003).

Conclusion

The present preliminary work demonstrated that amitraz has relatively conserved its tickicidal efficacy, both in vitro and in vivo, on both tested tick species than diazinon. A clue of tick insusceptibility to diazinon was noted suggesting a need to consider right application and choice of acaricides in order to avoid any resistance against ticks and in camels. Furthermore, avoidance of uncontrolled utilization of commercial insecticides and dependence on limited type of acaricides by pastoralists is recommended.

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Conflicts of interest

The authors have none to declare.

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