**Identification of Hard Tick Species, Modeling of Habitat Suitability and Evaluation of the Effectiveness of Selected Acaricidal and Herbal Preparations in Waglasta**

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Abstract

Cross-sectional and randomized controlled study designs were conducted from April 2020 to June 2021 to explore tick infestation prevalence, the association between the risk factors; and to evaluate the acaricidal efficacy of selected drugs commonly used in Waghimra, Ethiopia. A total of 384 animals screened for the presence of hard tick given a 52% overall prevalence of tick infestation. The genus *Rhipicephalus* (52.5%) was the most prevalent tick followed by *Ambyloma* (40.7%), while *Hyaloma* (6.9%) was the least familiar genus of ticks at the study site. In this study nine tick species were identified which including *A.cohaereneces* (9.9%), *A.variegatum* (31%), *H.marginatum* (1%), *H.rufipus* (1%), *H.truncatum* (5%), *R.decoloratus* (10.3%), *R.evrtsi evertsi* (9.3%), *R. pravus* (31%) and *R.pulchellus* (2%) in the study areas. The study also indicated that the ears (41.2%) and perineum (27.9%) found the most severely infested parts of the body, while the groin (10.3%), back/ legs (9.8%), chest (7.8%), and muzzle (2.9%) were rarely affected. The genus Rhipicephalus crowded in and around the ears (30.4%) and the perineum (13.7%) whereas Ambyloma and Hyaloma distributed fairly on all parts of the body. Animals with good body condition scores were found mostly disturbed (50%) than medium (28.43%) and weak (21.57%) animals. The Chi-square test suggested Sex and BCS (p<0.02), predilection site and district (p=0.00) had a statistically significant effect on the distribution of tick species among the risk factors. About 120 adult female ticks (*A.variegatum*) were used to conduct the acaricidal effectiveness of Amitraz and Diazinon. Maximum and minimum oviposition inhibition effects of 98.53% 86.67%, 73.55%, and 67.21% were registered by Amitraz and Diazinon respectively. Besides, Amitraz treatment found 58% of fatality and produced only a few numbers eggs whereas Diazinon was given 37% of mortality rates and ticks laid some batches of eggs within 7 days of incubation. However, there were no dead ticks of water-treated and were able to lay large quantities of eggs. Independent t-test showed that Amitraz and Diazinon had (92.80%) and (69.72) of mean percent of control. Analysis of variance showed that there exists a statistical significance (P=0.00) between the two chemical preparations. Despite no guidelines or rules on how to market, evaluate, prepare and use; consideration must be given to avoid the uncontrolled use of commercial insecticides and herdsmen reliance on limited types of acaricide. According to this study, Diazinon, commonly used by government veterinary clinics, has been shown less effective in treating and controlling tick population and therefore guarantee for further investigation, professional intervention, and government attention

**Keywords;** Acaricides, Adult Immersion Test, Hard Tick, Percent of Control, Oviposition Inhibition, Waghimra

**Introduction**

Although Ethiopia owned the largest livestock population in Africa, the contribution of the livestock sector in terms of supporting the country’s economy is lower than the expected massive potential. However, the sector contributed to foreign currency earnings through the export of live animals, meat, offal, partial and complete finished leather, and leather products. Diseases are among many constraints that hinder the proper use of this resource to endeavor food insecurity (CSA, 2020). Ticks are undoubtedly the chief organisms to hurt domestic animals with blood-feeding capacity (Bellgard et al., 2012). About 80% of the world's livestock population is exposed to ticks and tick-borne diseases(Kouam & Dongmo, 2018). In tropical Africa, ticks and tick-borne diseases are important diseases that challenge the livestock industry (Gashaw et al., 2018). Amongst the crisis caused by tick infestation are tick-borne morbidity and mortality, damage on hide and skins, enormous losses in growth, milk and meat production, costs of tick control, and prevention measures. Besides, ticks play a major role in the transmission of pathogens from animals to animals such as viruses, bacteria, and protozoa. It is the second most common vector of human infectious diseases, after mosquitoes. An economic loss due to tick has been estimated; 2 billion US$ in Brazil, 100 million Australian$ in Australia; 48 million US $ in Mexico, and 32.7million US$ in Uruguay per year(Adane et al., 2018; Kouam & Dongmo, 2018; Lovis et al., 2013; Nogueira Domingues et al., 2012).

They are compulsory external parasites of all vertebrates, grouped into a class of Arthropod order *Acari* and two families, *Ixodidae* (hard ticks) and *Argasidae*, the soft ticks. Among the seven genera of hard ticks, *Ambyloma*, *Rhipicephalus* (*Boophilus*), *Haemaphysalis*, and *Hyaloma* have been common in Ethiopia (Gashaw et al., 2018). At least 800 species of ticks are known worldwide and around 47 are reported in Ethiopia (Bekalu Gerem Eskezia, 2016; Pegram, 1981). The soft tick *Argas persicus* reported in Ethiopia on livestock, traditional human houses and trees, and plays an important role as carriers of diseases, especially in poultry (Pader et al., 2012).

The control and prevention measures of tick infestation include; acaricide treatment with different application techniques, tick-resistant animals, tick vaccines, and management interventions. Chemical Acaricides such as synthetic pyrethroids, organophosphates, and Amitraz remain the mainstay of tick control measures. However, misuse, underdosing and excessive uses of these chemicals favor developing tick resistance (Rosado-aguilar et al., 2010). Resistance is generally first recognized as the failure of a drug to control parasitism but the formal definition of resistance is a shift in the target species susceptibility to a drug. The most frequently used techniques to detect resistance in cattle ticks are the adult immersion test (AIT), larval packet test (LPT), and larval immersion test (LIT). However, for the success of any tick management strategy, it is necessary to use a test that is practical, quick, economical, and reliable to detect the presence of resistance in the target population(Gashaw et al., 2018).

Although tick remains a challenge in Waghimra zone, to our knowledge, the details of tick species distribution and the factors influencing their occurrence have not been studied well so far. Hence, identification of the dominant tick species and the resistance against available drugs is critical for the control and prevention of ticks and tick-borne diseases. Besides assessment of the effects of tick infestation on animals and damage to production is essential (Tadesse & Sultan, 2014). This study aimed at (1) to identify the tick species infesting ruminants raised under extensive system in the Waghimra Zone, (2) to assess the association between the risk factors, and (3) determine the status of susceptibility or resistance of commonly used chemical preparations against the most widespread tick species in Waghimra Zone.

**2. Materials and Methods**

2.1. Description of the Study Area

The study was carried out in different agro-ecologies of Waghimra Zone in the regional states of Amhara, Ethiopia. Topographically, Waghimra is located at 12 23' longitudes 13 16' N and 38 44'and 39 21' E latitudes in the east of the country. The elevation of the area ranges from 989 to 4043m above sea level. Waghimra is characterized by unimodal and erratic annual rainfall patterns varying from 350 to 650 mm and recurrent drought. The Zone has eight woredas and two administrative towns. The main rain season (summer) starts from late June to early September in the high land and midland areas whereas it is from early July to mid of August in lowlands(Adane et al., 2018).

2.2. Study Animals

The target populations were animals, regardless of species, kept in an extensive management system and heading to vet clinics from around during the study period. Information pertaining to individual animals such as age, sex, body condition, district with GPS coordinates and the species were recorded. Animals were classified into three age groups; young (<1years old) adult (<5years old) or old aged (≥5years old). Based on their body condition scores they were categorized as poor medium and good (Adane et al., 2018). Since there was no previous related study in the area; with 95% confidence interval (CI) at 5% desired precision and considering 50% of prevalence, the sample size has been calculated as

N =

Where N is number of total samples needed, P is expected disease prevalence, and D is the margin of error. Therefore, the calculated sample size for the study was 384 animals.

2.3. Study Design, Sample Collection, and processing

A cross-sectional study design was carried out from April 2020 to June 2021 to quantify tick infestations in animals at the study site. Sample collection format was prepared to record individual animal species, sex, age, predilection site from samples taken, body condition score, and district with GPS coordinate. Adult ticks feeding on animals carefully detached and placed in universal bottles with holes to allow for air circulation, and then preserved in 20 ml of 70% ethanol. Samples were immediately sent to Sekota Dryland Agricultural Research Centre Veterinary Laboratory (SDARCVL) after collection and kept at +40 ° C for further investigations. Identifications of collected samples were initially carried out to screen all samples with the help of a stereomicroscope, identification keys, and color print images of various tick species.

A controlled experimental design was carried out to evaluate the egg-laying inhibitory actions of selected chemical preparations on female *A.variegatum*. Engorged female ticks collected from animals were presented to clinics and natural pastures at Dehana district and then placed in a plastic bottle labeled with the sample code, date, and location. The animals we used for the experiment had not been received acaricidal treatment for a month before sample collection.

2.3.1 Evaluation of the Effectiveness of Selected Acaricides against *A.variegatum*

The test Acaricides were Amitraz 12.5% (made by Hebei Veyong Animal Pharmaceutical Co. China) And Diazinon 60% (made by Kafer El Zayat Pesticides & Chemicals Co., Egypt) gained from the veterinary pharmacy of Sekota Dryland Agricultural Research Centre. Diazinon and Amitraz were diluted in distilled water at concentrations of 1: 1000 and 1.6: 1000 with a final volume of 1000 ml according to the manufacturer's instructions on the package insert. We used the Adult Immersion Test technique to evaluate acaricidal effectiveness as recommended by Drummond et al. (1973) and modified by FAO (2004) and the South Africa Bureau of Standards, South Africa (D. Ayana et al., 2014)

Ticks were washed with water to remove feces, eggs, other debris; allowed to dry on a paper towel, and then divided into groups (10 ticks each group) and weighed. The grouped ticks were immersed in 20 ml of each acaricide and the control with three repeats and stirred vigorously for one minute in a 100 ml plastic container. The acaricide poured off through the sieve; the ticks were then stored in plastic containers before removal and carefully dried on absorbent paper. Afterward, all treatment and control groups were placed in a Petri dish and stuck (ventral side up) with double-sided adhesive tape. Subsequently, Plates were placed in a larger plastic box and incubated at 27 ° C with a moistened sponge inside. After 7 days of incubation, the number of live or dead ticks was counted, and the eggs produced by each treatment group had weighed. The oviposition responses and mortality were watched at regular intervals. Acaricides efficacy was estimated using the Egg-Laying Test (ELT) in which the egg mass of acaricide-treated ticks and the egg mass of water treated ticks were compared, and finally, the percentage control value was estimated (D. Ayana et al., 2014)

Percent control = x 100

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

Resistance (%) = 100 - control%

**3. Data Management**

The data were entered in a Microsoft Excel spreadsheet and analyzed by the SPSS 25 program (2017). Descriptive statistics summarised the distributions of hard ticks in the study sites and each point percentage was calculated as the animals in each category were divided by the total number of tick-infested animals multiplied by one hundred. The overall prevalence of tick infestations is calculated as the sum of positive animals divided by the total number of animals examined multiplied by one hundred. The associations between various possible risk factors and tick infestation were analyzed using a logistic regression model. The chi-square test was used to assess the degree of association between tick infestation and risk factors. An independent t-test was used to examine the mean percent control value used to compare acaricidal effectiveness. Analysis of variance (ANOVA) was used to examine the association between oviposition responses of the test acaricides. Besides, a P-value less than 0.05 was considered significant at 95% confidence interval.

**4. Results**

This study found that out of 384 animals examined, 200 (52%) were found infested by various ticks. About 59.7% of goats, 39.4 % of cattle, and 52.3% of sheep were found infested with various degrees of severity. The study identified three genera and nine species of ticks with *A.variegatum* as the most abundant (Table1). A group of 120 adult female swollen ticks collected and egg-laying test of Amitraz and Diazinon showed the mean oviposition inhibition control percent of 92.80% and 69.72% (Table4).

**Table 1:** Descriptive statistic on the distribution of tick genera and logistic regression analysis of possible risks in the study area

|  |
| --- |
| VariablyN *Ambyloma (%) Hyaloma (%) Rhepicephalus (%)* P-value *(CI In.vals)* |
| |  |  |  |  |  |  | | --- | --- | --- | --- | --- | --- | | **Species** | Goat 231 | 50 24.5 | 5 2.5 | 83 40.7 0.06 -0.72-0.36 | | | Cattle 109 | 24 11.8 | 8 3.9 | 11 5.4 | | | Sheep 44 | 9 4.4 | 1 0.5 | 13 6.4 | | | **Bcs** | Poor 132 | 19 9.3 | 3 1.5 | 22 10.8 0.02 0.11-0.77 | | |  | Medium 191 | 28 13.7 | 2 0.9 | 28 13.7  57 27.9 |  | |  | Good 71 | 36 17.6 | 9 4.4 | | **Sex** | Male 168 | 39 19.1 | 9 4.4 | 34 16.7 0.02 0.15-1.69 | | |  | Female 216 | 43 21.1 | 5 2.5 | 73 35.8 | | | **District** | Dehana 102 | 29 14.2 | 6 2.9 | 20 9.8 0.00 0.87-1.99 | | |  | Sekota 185 | 52 25.5 | 6 2.9 | 39 19.1 | | | **Age**  **Presite** | Aberg 97  Young 48  Adult 121  Old 35  Ear 384  Chest 384  Inguinal 384  Leg 384  Muzzle 384  perineum 384 | 2 1.0  18 8.8  49 24.0  16 7.8  20 9.8  10 4.9  14 6.9  14 6.9  3 1.5  22 10.8 | 7 3.4  2 1  9 4.4  3 1.5  2 1.0  2 1.0  2 1.0  1 0.5  0 0.0  7 3.4 | 48 23.5 0.29 -0.99-0.29  27 13.2  64 31.4  16 7.8  62 30.4  4 2.0  5 2.5 0.00 0.19-0.23  5 2.5  3 1.5  28 13.7 | | |

*N=Number Os Animals Tested \*CI= Confidence Intervals\* BCS=Body Condition Scores Presite= Predilection Site*

Table 2: Distributions of tick infestation in the study areas by species.

|  |  |  |
| --- | --- | --- |
| **Tick species** | **Sekota Dehana Abergelle total (%)** |  |
| ***A.cohaereneces***  ***A.vartiegatum***  ***H.marginatum***  ***H.rafipues***  ***H.truncatum***  ***R.decoloratus***  ***R.evrtsi***  ***R.pravus***  ***R.pulchus***  **Overall** | 7 12 1 20(9.8%)  45 17 1 63(31%)  0 2 0 2(1%)  2 0 0 2(1%)  5 4 1 10(5%)  1 1 19 21(10.2%)  15 2 2 19(9%)  20 16 27 63(31%)  3 1 0 4(2%)  98(48%) 55(27%) 51(25% 204(100%) | |

Table 3: Mean oviposition of female *A.variegatum* and the mean %control value of each trials after immersion in Amitraz 12.5% ​​and Diazinon 60% EC.

|  |  |
| --- | --- |
| **Trial Treatment N M1 S M2 % C** | |
| **1** Amitraz 10 20.26 5 0.11 89.57  Diazinon 10 24.59 7 0.34 67.28  Water 10 23.7 10 1.05 0  2 Diazinon 10 19.07 6 0.26 70.83  Amitraz 10 19.23 3 0.01 98.53  Water 10 18.39 10 0.91 0  3 Water 10 21.54 10 0.92 0  Amitraz 10 20.44 4 0.12 86.67  Diazinon 10 20.85 7 0.30 67.21  4 Diazinon 10 19.56 5 0.27 73.55  Amitraz 10 19.81 5 0.04 96.45  Water 10 20.29 10 1.00 0 |

*N=total number of engorged ticks, m1=average mass of engorged ticks in gram, S= average ticks survived,*

*M2= average mass egg laid in gram, %C =average percent control*

Table 4: T-test analysis of mean% of oviposition inhibition by Amitraz and ​​Diazinon against *A. variegatum*

|  |
| --- |
| Acaricide N Mean %C SD t-value df 95% CI |
| Amitraz 4 92.80 5.61 7.23 6 15.27-30.91  Diazinon 4 69.72 3.06 | |

*%C=Percent control; N= Number of trials; SD=Standard Deviation; NS= Not significant; DF =degree of freedom;*

*CI = of interval*

Table 5: Overall mean percent oviposition control of Amitraz 12.5% and Diazinon 60%EC at field recommended concentration against adult female

|  |
| --- |
| Treatment minimum efficacy maximum efficacy mean efficacy±SD |
| Amitraz 86.67 98.54 92.80±5.61  Diazinon 67.21 73.55 69.72±3.06 |

Table 6: Analysis of variance of the effects of Amitraz 12.5% and Diazinon 60% EC against oviposition response of A. variegatum.

|  |
| --- |
| Sources of Sum of Square DF Mean Square F Sig.  variation |
| Between Groups 1.75 2 0.875    Within Groups 0.026 9 0.003 301.431 0.00  Total 1.78 11 |

*DF =degree of freedom*, F=Value, Sig= significance

**5. Discussion**

Ticks, tick-borne diseases, and trypanosomiasis are diseases that have caused devastating economical losses in the livestock industry in tropical Africa. The effects of a tick infestation are a drastic decrease in growth, milk and meat production, damage on hide and skins, disease transmission. Ticks are the second most common carriers of human infectious diseases after mosquitoes(Adane et al., 2018; Gashaw et al., 2018; Kouam & Dongmo, 2018; Lovis et al., 2013; Nogueira Domingues et al., 2012).

In this study, the overall tick infestation prevalence of 52% was comparable to the report of 59.4% (Tadesse & Sultan, 2014) in Fiche Selale, Oromia, Ethiopia, and lower than the report of 75.5% by (Kemal et al., 2016)in Arbegona, Southern Ethiopia, 83.3% by (Nasero & Roba, 2020)in Itang, Gambella, and 89.89% by (M. Ayana et al., 2021)) in Yabello, Oromia, Ethiopia. Contributing reasons for this deviation could be the study site differences, sample size inconsistencies, management practices, animals movement in search of watering and grazing during the dry season, using insecticides, and other preventive measures (Tadesse & Sultan, 2014).

The genus *Rhipicephalus* was the most common tick (52.5%), followed by *Ambyloma* (40.7%), while *Hyaloma* (6.9%) was the least common at the study site. This study identified total of nine tick species including; *A.cohaereneces* (9.9%), *A.variegatum* (31%), *H.marginatum* (1%), *H.rufipus* (1%), *H.truncatum* (5%), *R.decoloratus* (10.3%), *R.evrtsi evertsi* (9.3%), *R. pravus* (31%) and *R.pulchellus* (2%) in the study (Table 2). *A.variegatum* found the most widespread tick species in the midlands of Sekota (45%), highlands of Dehana and (30.9%), and the least in the lowlands of Abergele (0.2%) districts. In the lowland of Abergele district, the species R.pravus dominated (52.9%) followed by R.decoloratus (37.3%).

The study also found the ears (41.2%), perineum (27.9%), severely infested, while the groin (10.3%), back and legs (9.8%), chest (7.8%), and muzzle (2.9%) was slightly affected body parts. The ears found the most disturbing body parts followed by the perineum and the groin (table 2). The genus Ambyloma and Hyaloma were found fairly distributed on the body of animals whereas Rhipicephalus crowded on the ears and the perineum. The rate of tick infestation was higher in female animals than in male animals (table 1). Animals with good body condition scores were found severely affected (50%) followed by medium (28.43%) and poor (21.57%) body conditioned animals (table1). An increased infestation rate was noted in animals with good and medium body condition scores. In fact Animals with good and medium body condition scores covering long distances in search of grazing and watering during drought, a pick time of tick population, contributed reasonably to increase the chance of contracting tick infestation. On the other hand, poor body conditioned animals kept at home or cover only small distances are given a lower chance of being infected. Districts, predilection site (p=0.00), sex, and BCS (p<0.02) had noted statistically significant while other risk factors were found insignificant (Table 1).

A controlled laboratory experiment showed the mean egg-oviposition-inhibition effect of Amitraz (92.80%) and Diazinon (69.72% ), which was lower than the report of 100% and 88.85% (D. Ayana et al., 2014) in Borena, Oromia, Ethiopia. The maximum and minimum mean oviposition inhibition effects by Amitraz were 98.53% and 86.67% whereas Diazinon counteracts 73.55% and 67.80% of oviposition. Amitraz and Diazinon revealed 92.72±5.61 and 69.72±3.06 percent of overall mean oviposition inhibition effects. Nearly 58% of the Amitraz-treated ticks found died, and the survived ticks laid no or few eggs (table 4) which were not comparable with the death report of 100% (D. Ayana et al., 2014) and 33.33% (Gashaw et al., 2018) against *A.gema* and *R.decoloratus* around Borena and Sebeta, Ethiopia. The experiment also showed a mass death of 38.7% of ticks treated in Diazinon and laid a small number of eggs which was not in line with the report of 100% (Gashaw et al., 2018). This could be because uncontrolled use of acaricides for a long time by the community might favor ticks to develop resistance. On the contrary, all water-treated ticks survived and produced large numbers of eggs (Table 3). Analysis of variance showed statistical significance on counter oviposition action between the two drugs against *A.variegatum*. Amitraz had a better acaricidal effect which was in line with the report by (Petros et al., 2015) in camels, Somalia, Ethiopia.

**6. Conclusion and Recommendations**

Ticks are undoubtedly the chief organisms to hurt domestic animals with blood-feeding capacity. Consequences of tick infestation include enormous losses in milk and meat production, damage on the skin and hide, and costs of tick control and prevention measures. Tick resistances develop from misuse, underdose, and excessive use of chemicals. This study identified three genera and nine species with different relative distributions. Amitraz showed a higher counter egg-laying and death response than Diazinon against *A.variegatum*. Despite no guidelines or rules on how to market, evaluate, prepare, and use, consideration must be given to avoid the uncontrolled use of commercial insecticides and shepherds' reliance on limited types of acaricide. According to this study Diazinon, commonly used by government veterinary clinics, has been shown less effective in treating and controlling tick population and therefore guarantee for further investigation, professional intervention, and government attention

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