Evaluation of Ethno-veterinary approaches to Control Ticks in Nepal

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ABSTRACT

Tick borne diseases such as Babesiosis and Theileriosis are common in Nepal. Farmers are using several plants as traditional ethnoveterinary medicines to control ticks in their animals. However, their efficacy have never been assessed. A study was conducted to evaluate the efficacy of plant extracts used in ethnoveterinary medicine by Nepalese farmers to control ticks. 100 rural farmers, 50 each from Tanahun and Nawalparasi districts, were interviewed through semi-structured questionnaires to document the ethnoveterinary plants used to control ticks. Among the 26 plants documented, Neem (*Azadirachta indica*) was used by 42 out of 100 respondents, Titepati (*Artemisia* spp.) by 34 respondents, Sisno (*Utrica dioica*) by 10 respondents and Bozo (*Acorus calamus*) by 6 respondents. The choice of plants used was determined by their local availability. Besides, mapping of livestock tick was conducted by collecting ticks from 21 districts. A total of five genera of ticks were identified viz. *Boophilus microplus, B. annulatus, Hyalomma marginatum issaci, Rhipicephalus haemaphysaloides, R. Sanguineus, Haemaphysalis bispinosa* and *Ixodes ovatus. Boophilus microplus* was found in all study districts and all the livestock species indicating the risk of enzootic stability of Babesiosis in local cattle. In a field trial conducted with 70 cattle at Nawalparasi district, Cypermethrin 0.02% (Clinar®) was found to be most effective in reducing the number of ticks (97.17% reduction) followed by 20% Neem extract (91.46% reduction) and 20% Bozo extract (89.88% reduction in ticks) ($p<0.05$). All the plant extracts exhibited more than 50% reduction in ticks at 3 days after treatment (DAT). Neem and Bozo extracts were found superior in tick control among the traditionally used plant extracts. Based on their local availability, Neem in Terai and Bozo in both Terai and Hills might be effectly used for the control of livestock ticks in Nepal in an eco-friendly manner.

Keywords: *Eco-friendly, Plant extracts, Tick control*

INTRODUCTION

Ticks, the haematophagus parasites, are the most common external parasite of economic importance in the small holder farming system of Nepal. High mortalities are reported in cattle due to ticks and tick borne diseases in Africa and across the tropical areas (Muchenje *et al.,* 2008). Ticks transmit a wide variety of pathogenic agents than other groups of arthropods (Oliver 1989), Babesiosis, Theileriosis, Trypanosomiasis and Ehrlichiosis are some of the examples of tick-borne livestock diseases which cause morbidity and mortality in animals. Besides, ticks cause tick worry,

blood loss and damage to skin, hides, and teats leading to formation of wounds predisposing animals to secondary bacterial infections. (Peter *et al.,* 2005).

Ticks are commonly controlled using conventional acaricides however, it has certain shortcomings. They are expensive and not readily available to rural farmers. They are toxic to livestock and humans, their residues remain in animal tissues and are harmful to the environment. Another important threat is that, ticks are developing resistance against a range of conventional acaricides available in the market. Due to these shortcomings of the conventional acaracides, resource-limited farmers are reverting to alternative methods and remedies that fall within ethnoveterinary practices. One of such method is the exploitation of eco-friendly, economical, safe and effective indigenous plants. These plants are the miraculous laboratories of nature that provide various kinds of molecules. Plant extracts provide valuable alternatives to and complements conventional veterinary medicine and is increasingly evident in the western world where herbal medicine and other alternative approaches are becoming the mainstream practices. Ethnoveterinary practices are of specific value in developing countries where allopathic medicines are often beyond the reach of livestock producers. In Nepal, farmers are using several traditional plants that include Neem (*Azadirachta indica*), Titepati (*Artemisia* spp.), Sisno (*Utrica dioica*), Bozo (*Acorus calamus*) and several other plants to control ticks, the choice of plants largely driven by local availability. However, their efficacy have never been assessed. This study was conducted to understand the common plants farmers are using to control the ticks and to measure their efficacy against commonly found livestock ticks in Nepal.

MATERIALS AND METHODS

The research was conducted from 15th July 2011 to 15th October 2011. *In-vitro* trials were conducted at Parasitology laboratory at Animal Health Research Division (AHRD), Nepal Agriculture Research Council (NARC). In-vivo trial was conducted at Gaidakot Municipality, Nawalpur District. For questionnaire survey an open-ended questionnaire was prepared. Survey on the existing practices on ectoparasite control and use of plants for tick control was conducted in Tanahun (Hilly district) and Nawalparasi (Terai district). Altogether 100 farmers were interviewed focusing on the ethnoveterinary remedies for tick control.

Mapping of livestock tick

Ticks were collected from the naturally infested animals of 21 districts of Nepal regardless of age, sex and ecological region. The districts from where ticks were collected include Jumla, Okhaldhunga, Kaski, Lalitpur, Kathmandu, Baglung, Myagdi, Bhaktapur, Arghakhachi, Morang, Sunsari, Jhapa, Siraha, Saptari, Dhanusha, Nawalparasi, Rupendehi, Chitwan, Bara, Parsa and Udyapur (Figure 1). Ticks were hand-picked from different parts of the body viz. ear, dewlap, tail base, foreleg, hindleg, udder, inguinal region, perennial region etc. The ticks were immediately preserved in vials containing 5% glycerine in 70% alcohol. Ticks containing vials were labeled and transported to AHRD, NARC for further identification. The preserved ticks were examined for their morphological characteristics under sterio-microscope and identified according to the figures and keys described by acarology division (Kaiser, 1987 and Morel, 1989).

Preparation of plant extracts

Four plants namely; *Azadirachta indica* (Neem), *Acorus calamus* (Bozo), *Artemisia vulgaris* (Titepati) and *Utrica dioica* (Sisno) were used in the trial. Leaves of Neem, rhizomes of Bozo collected from Chitwan District and the leaves and soft aerial parts of Sisno and Titepati collected from Kavre district were taken to AHRD, NARC, Khumaltar, Lalitpur. Aqueous extracts having concentrations of 20%, 10% and 5% were prepared and stored in a refrigerator at 4˚ C until used in the experiment.

In-vivo trial

In-vivo trial was conducted at Gaidakot Municipality, Nawalpur District. Altogether 70 cattle, naturally heavily infested with ticks were selected. The animals were of different breeds, colour, age, sex, parity and body conditions. One square foot area at three distinct regions in the body was marked at dewlap, tail-base and udder respectively. The total number of ticks present on the selected areas were counted. 12 different solutions containing aqueous extract of Neem, Sisno, Bozo and Titepati having 5%, 10% and 20% were used in the test. Distilled water and Cypermethrin 0.02% (standard) were used as negative and positive control respectively. Altogether 14 treatments with 5 replications each were made. Test solution was applied on the demarcated area with paint brushes after counting the number of ticks on the first day. The number of ticks remaining on the site was counted on 2nd, 3rd and 4th day of application of the extracts. The percentage reduction in number of ticks was then analysed using MSTAT-C, computer software program.

RESULTS

Documentation of plants used in ethno-veterinary practices to control ticks

Altogether 26 plants and plant parts were documented (Table 1). We found that Neem (*Azadirachta indica*) was the most used plant for tick control. Ten out of fifty respondents in Hilly region and 32 out of 50 respondents in Terai region used Neem for tick control. Titepati (*Artemisia* spp.) was used by 34 farmers (26 out of 50 farmers in hilly region and 8 out of 50 farmers in terai). This indicates that these plants are common to both the highland areas and low land areas.

Table 1. List of plants used to control ticks

Common name	Scientific name	Number of respondents using plants as a method of tick control		
		Tanahun	Nawalparasi	Total
Neem	*Azadirachta indica*	10	32	42
Titepati	*Artemisia japonica*	26	8	34
Banmara	*Ageratina adenophora*	21	6	27
Sisno	*Utrica dioca*	8	2	10
Lemon extract	*Citrus limon*	7	3	10
Gandhe	*Ageratum hausronianum*	5	5	10
Salt	Sodium chloride	6	3	9
Tulsi	*Ocimum tenuiflorum*	3	6	9

Tobacco	*Nicotiana tabaccum*	-	7	7
Bojo	*Acorus calamus*	3	3	6
Haldi/ Besar	*Curcuma longa*	2	3	5
Ghiukumari	*Aloe vera*	2	3	5
Asuro	*Adhatoda vasica*	4	-	4
Timur	*Zanthoxylum armatum*	2	2	4
Bakaino	*Melia azadirach*	-	4	4
Mustard cake	*Barassica campestris*	1	2	3
Tejpatta	*Cinnamomum tamala*	2	-	2
Amala	*Emblica officinalis*	2	-	2
Guava leaves	*Psidium spp.*	1	1	2
Garlic	*Allium sativum*	1	1	2
Simali	*Vitex negundo*	1	-	1
Jangli tulsi	*Croton bonplandianum*	1	-	1
Cow butter oil	-	1	-	1
Chilaune bark	*Schima wallichii*	1	-	1
Bhojpatra	*Betula utilis*	1	-	1
Zeera	*Cuminum cyminum*	-	1	1
Peach leaves	*Prunus Persica*	-	1	1

Tick mapping

From 21 study districts, the total numbers of ticks examined were 1268, of which 1001 were identified as female, 216 as male and 57 as nymphs. A total of five genera of ticks were identified from livestock in 21 districts (Table 2).

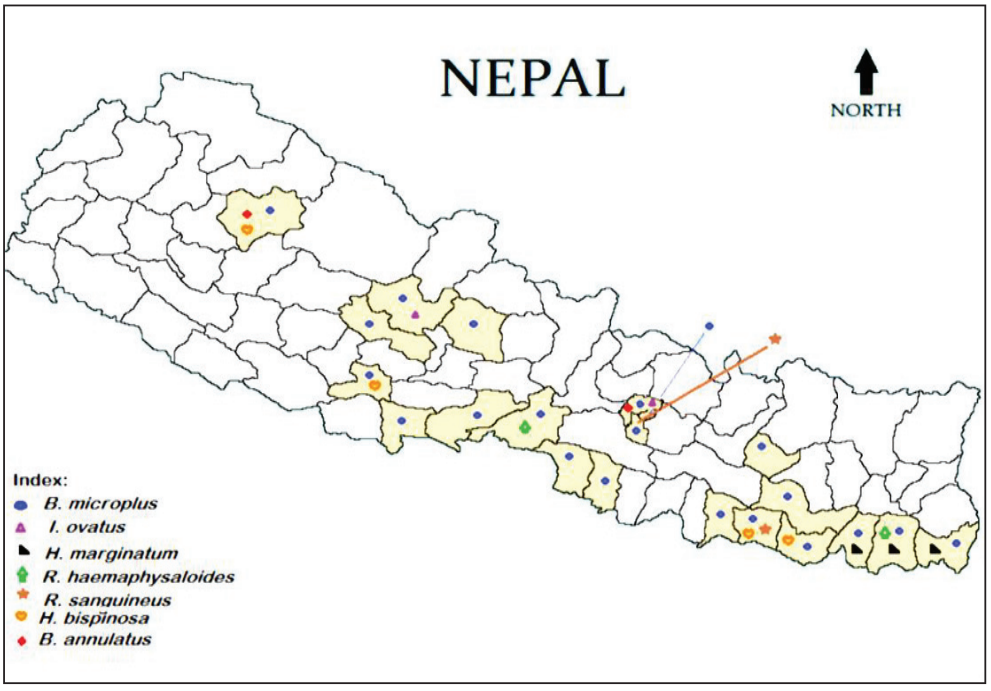

Figure 1. Map of sampled districts and different ticks found in those districts

In-vivo trial

At 1st day after treatment (DAT), all the treatments were significantly different as compared to the standard (Cypermethrin 0.02%). Among the plant extracts, the highest percentage reduction in number of ticks was recorded in 20% Bozo extract solution with a mean reduction of 58.05% and the least was recorded in 5% Neem extract solution with a mean reduction of 21.52%. No statistically significant difference in the percentage reduction in ticks in cattle treated with Bozo 20% extract, Neem 20% extract and Titepati 20%.

Table 2. Ticks species identified

Tick species	Districts	Livestock species
Boophilus microplus	All	Cattle, buffalo, goat, rabbit, pig
Boophilus annulatus	Jumla, Kathmandu	Cattle
Hyalomma marginatum issaci	Morang, Sunsari, Jhapa	Cattle
Rhipicephalus haemaphysaloides	Morang, Chitwan	Cattle
R. sanguineus	Dhanusa, Lalitpur	Cattle, Goat
Haemaphysalis bispinosa	Jumla, Argakhachi, Siraha, Saptari	Cattle
Ixodes ovatus	Myagdi, Kathmandu	Cattle, Goat

At 2nd DAT, all the treatments were significantly different as compared to the standard (Cypermethrin 0.02%) which has the highest percentage mean reduction in the number of ticks (93.10%). Among the different plant extracts, 20% Neem extract has the highest percentage reduction in number of ticks (mean 74.48%) at $P<0.05$. There was no significant difference in percentage reduction in number of ticks between 20% Neem extract and 20% Bozo extract solution. The result of these two extracts was at par with 20% Titepati extract solution, 20% Sisno extract solution and 10% titepati extract solution. On the 3rd DAT, the highest percentage reduction in number of ticks was found in standard (Cypermethrin 0.02%), having mean reduction of 97.17%. Among the plant extracts, 20% Neem extract had the highest mean percentage reduction in number of ticks (91.46%). There was no statistically significant difference between 20% Neem extract (mean percentage reduction in number of ticks 91.46%) and 20% Bozo extract (mean percentage reduction in number of ticks 89.88%) at $P<0.05$. Likewise, 20% Titepati extract solution (mean percentage reduction in number of ticks 85.85%) was significantly lower than the above two treatments. All the plant extracts exhibited mean percentage reduction in number of ticks by more than 50% until 3rd day after treatment. The study revealed that, the highest efficacy was found in 20% Neem extract and 20% Bozo extract (Table 3).

Table 3. In-vivo efficacy of different plant extracts

Treatment	Percentage reduction in number of ticks (Days post treatment)		
	Day 1	Day 2	Day 3
5% Neem Extract	21.52^{h} (27.56)	36.45^{f} (36.84)	64.89^{f} (53.75)
10% Neem Extract	31.20^{efg} (33.86)	49.18^{e} (44.51)	73.63^{de} (59.23)
20% Neem Extract	56.5^{b} (48.79)	74.48^{b} (59.79)	91.46^{b} (73.39)
5% Sisno Extract	25.86^{gh} (30.50)	50.49^{e} (45.27)	59.24^{g} (50.30)
10% Sisno Extract	35.50^{def} (35.33)	57.81^{de} (49.48)	67.04^{f} (54.95)

20% Sisno Extract	39.21^{cd} (38.74)	71.28^{bc} (57.59)	75.90^{d} (60.59)
5% Bozo Extract	28.73^{fg} (32.35)	52.54^{e} (47.52)	65.57^{f} (54.06)
10% Bozo Extract	40.52^{c} (39.50)	64.33^{cd} (53.33)	74.04^{de} (59.35)
20% Bozo Extract	58.05^{b} (49.62)	72.40^{b} (59.51)	89.88^{b} (71.67)
5% Titepati Extract	27.39^{fg} (31.40)	57.49^{de} (49.32)	68.90^{ef} (56.09)
10% Titepati Extract	36.87^{cde} (37.20)	67.39^{bc} (55.34)	73.81^{de} (59.20)
20% Titepati Extract	53.40^{b} (46.93)	71.59^{bc} (58.29)	85.85^{c} (67.88)
Negative control	4.02^{i} (9.660)	12.53^{g} (11.32)	11.86^{h} (13.12)
Positive control	80.75^{a} (64.04)	93.10^{a} (75.88)	97.17^{a} (80.95)
LSD (P=0.05)	3.843	5.149	3.337
SEM±	1.354	1.814	1.176
CV%	8.07%	8.07%	4.52%
Grand Mean	37.535	50.300	58.180

CV: Coefficient of variation, LSD: Least Significant Difference
Value with the same letters in a column is not significantly different at 5% by DMRT
The figures in parentheses are arcsine transformation.

DISCUSSION

Of the five genera, *Boophilus microplus* was the most abundant tick in all study districts and in all livestock species. It is likely that high infection rates of *B. microplus* ticks could maintain enzootic stability of Babesiosis in local cattle. In this study, almost in all animals, the number of tick infestation per animal was more than 100, *Hyalomma marginatum issaci*, the vector tick for theileriosis, was identified from three eastern Terai districts. Eastern Terai region is endemic for Theileriosis because of the presence of both pathogen and vector tick (Shrestha, *et al.,* 2005). *Haemaphysalis bispinoa,* a vector tick for *Theileria sergenti*, which is only common in Japan and Korea, may also transmit the disease, however, this particular protozoan parasite has not been identified from Nepal (Shrestha *et al.,* 2005). *Ixodes ovatus* tick was identified from Myagdi and Kathmandu from cattle and goat respectively.

Among the plant extracts, neem leaves extracts significantly had higher mean reduction of ticks. This finding is in line with Rahman *et al.* (2009) who found a mean reduction of 68% tick when 15% neem extract was sprayed on the calves in a study conducted in Bangladesh. 8% concentration of neem seed extract had the highest mortality compared to neem bark extract and 8% neem leaves extract, (Ghosh et al. 2010). Ismail *et al.* (2002) reported a high neem oil concentration of 40%, which was very much effective against *R. pulchellus* larvae. Choudhury (2001) reported 100% mortality of the *R. sanguinus* larvae at 8, 6, 4, and 2 hour after treatment with neem seed oil at the concentration of 20%, 40%, 60% and 80% respectively. In our experiment the 20% aqueous extract of neem leaves showed 91.46% reduction in tick 3 days after the application. Similar results with 92.4% mortality of ticks on third day post treatment with 8% concentration ethanolic extract of neem seed extract was recorded by Ghosh *et al.* (2010) in a study conducted in India. The 90-100% efficacy of cypermethrin was earlier reported by Khan (1996) against B. microplus in cattle along with residual period of 8-15 days. Main arthopodicidal neem compounds normally require a longer time to show their relevant properties. All the plant extracts showed a time and concentration dependent effect on the ticks. The reduction in the number of ticks may be either

from the death, from repellent property of the herbal drugs, from anaesthetization and dropping off from the body or combined effects of herbal drugs (Shrestha *et al.,* 2005).

Among 135 described compounds, Azadirachtin A (AZA) commonly referred to as azadirachtin stands out (Liu *et al.,* 2005). Azadirachta indica is considered the most phytochemical source of pesticides, and is widely used all over the world (Forster and Moser, 2000). In fact, the active compounds of neem show many modes of action against arthropods, such as antifeedant activity, growth regulation, repellency, reduction of fecundity and oviposition, changes in biological fitness, and blocked development of vector-borne pathogens (Mulla and Su, 1999; Schmutterer, 1990a). In a similar research Ghosh et al. 2010 found that the extract prepared from rhizome of *Acorus calamus* proved highly efficacious and 100% final mortality within 14 days post treatment. The extract was found safe and no reaction was observed when animals were treated with 50% concentration (Ghosh *et al.* 2010) which is 2.5 times the concentration used for in vivo studies in present research.

CONCLUSION

Neem leaves are used by majority of the farmers as a means of ethnoveterinary remedy to control ticks in Nepal. *Boophilus microplus, B. annulatus, Hyalomma marginatu issaci, Rhipicephalus haemaphysaloides, R. Sanguineus, Haemaphysalis bispinosa* and *Ixodes ovatus* are the major tick species found in Nepal with *B. microplus* present in all the districts and in all the livestock specie. 20% aqueous extract of Neem (*Azadirachta indica*) is found to be the most effective remedy for ticks in *in-vivo* trial followed 20% aqueous extract of Bozo (*Acorus calamus*). Hence, Neem in Terai and Bozo in both Terai and Hills can be used to control ticks without any harm to the animals. For further studies we suggest to analyse the chemical constituents of aqueous extracts and quantify them to use for tick control in livestock.

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