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PLANT EXTRACTS AS ALTERNATIVES TO ANTI-ANTHELMINTIC DRUGS: FINDINGS FROM MAEDWANG SUBDISTRICT, THIMPHU

NIDUP DORJI* AND PHUB DORJI

Department of Animal Science, College of Natural Resources, Royal University of Bhutan, Lobesa, Punakha

*Author for correspondence: nirinjigpel17@gmail.com

ABSTRACT: A study was conducted to investigate the prevalence of endo-parasites in dairy cattle and evaluate anti-anthelmintic properties of common wormwood (*Artemisia vulgaris*) leaves, papaya (*Carica papaya*) seed and mustard (*Brassica nigra*) oil. The plants were collected, sun dried, pulverized and mixed with warm water to prepare plant suspensions. The suspensions were administered to 3 groups of cattle, with each group consisting 10 cattle. Out of 104 fecal samples analyzed, about 65.38% were positive, including *Strongyles* 2.88%, *Ascaris* 39.42%, *Coccidia* 11.53%, *Fasciola* 0.96% and mixed infection 10.57%. All plants suspensions were effective in reducing the egg count but mustard oil proved to be more effective. The study concluded that all three plant suspensions were effective against endo-parasite and have potentials to enhance milk production.

Keywords: Anti-anthelmintic; common wormwood; mustard oil; papaya seed; plant suspension.

1. INTRODUCTION

Agriculture is the backbone of rural economy. Over 62% of Bhutanese population are engaged in farming activities (PPD 2013). Farmers rear livestock of different breeds and livestock contributes about 3.79% to the total Gross Domestic Product (DoL 2012). Livestock provides a sense of security to rural farmers in times of crop failure since they can be exchanged readily for cash or food grain. Livestock, especially dairy cattle are reared under semi-extensive systems where they graze freely in forest and community grazing land. This system exposes cattle to parasites and other communicable diseases. Terbalanche (1979) attributed the problem to more number of parasitic cases emerging due to poor pasture grazing management system, hampering production capabilities and health of livestock. Due to parasitic problem, high numbers of synthetic de-worming drugs are being used incurring huge budget expenditure. Similarly, in Bhutan, a huge amount of budget is spent on procurement and usage of synthetic deworming drugs. As per the record of National Center for Animal Health, Serbithang, there has been an expenditure of over Nu. 8 million in three consecutive years from 2012 to 2014. With high usage of deworming drugs, issues of drugs resistance are being reported (Manthri et al. 2011). It calls for alternative methods to address the issue. One of the alternatives could be the use of plant products. Some plants are reported to have potential to combat the prevalence of parasite in cattle since it has anti-anthelmintic properties. There are evidences from India, South Africa and China on the use of these plants as alternate to synthetic anti-anthelmintic drugs (Ameen et al. 2010).

In Bhutan, there is traditional practice of using plants to treat diseases. However, there is lack of scientific evidence on the use of certain plants for treatment of diseases. Therefore, a study was conducted with the main objectives to investigate the prevalence of endo-parasites infestation in cattle and evaluate the efficacy and anti-anthelmintic properties of Artemisia leaf and papaya seed in the treatment of endo-parasites. These plants could be an alternative to synthetic drugs and minimize the use of synthetic deworming drugs.

2. MATERIALS AND METHOD

2.1 Study site

Maedwang subdistrict under Thimphu district was selected for the study (Figure 1). The subdistrict was selected because it had the highest number of endo-parasitic cases among subdistricts, as per the clinical records of District Veterinary Hospital. The study was conducted in Tsalluna under Maedwang subdistrict located in the south-east of Thimphu (Figure 1). Tsalluna comprises of 17 villages with an area of 223.24 sq. km (NFYP 2012). The subdistrict is enriched with fertile soils that are good for agriculture farming. Tsalluna was selected because it has large number of dairy groups and cattle population. The subdistrict has about 22,149 cattle heads that include 208 local cattle with more number of female cattle (145 numbers) and 21,941 numbers of improved cattle (21,891 female cattle) (DoL 2013).

2.2 Experimental design and treatment

The experiment was conducted using the Completely Randomized Design (CRD). The experimental treatments were plant suspensions of common wormwood (*Artemisia vulgaris*) leaves (T1), papaya (*Carica papaya*) seed (T2) and mustard (*Brassica nigra*) oil (T3). The Draw Lots method was used for sampling 66 infected cattle. Of these, 38 cattle had no infestation. Only 30 endo-parasite infected dairy cattle were randomly selected and tied in one place for identification of dung patches. The cattle were divided into three groups with each group consisting 10 cattle. The plant suspensions were administered to the groups.

2.3 Cattle sampling and dosage administration

The animals were administered with suspensions of common wormwood leaves, papaya seed, and mustard oil. The sample with initial egg count was used as control. Dosage for each herbal plant extract was administered at 200 g in 500 ml of warm water per adult cattle and mustard oil was administered at 200 ml per cattle. Fecal samples from treated animals were collected after every one week for egg count assessment. The samples were collected in 30g plastic vials in the morning from the freshly defecated patch. The samples were analyzed using stoll, sedimentation and floatation methods, to detect different types of worm prevalence and egg count.

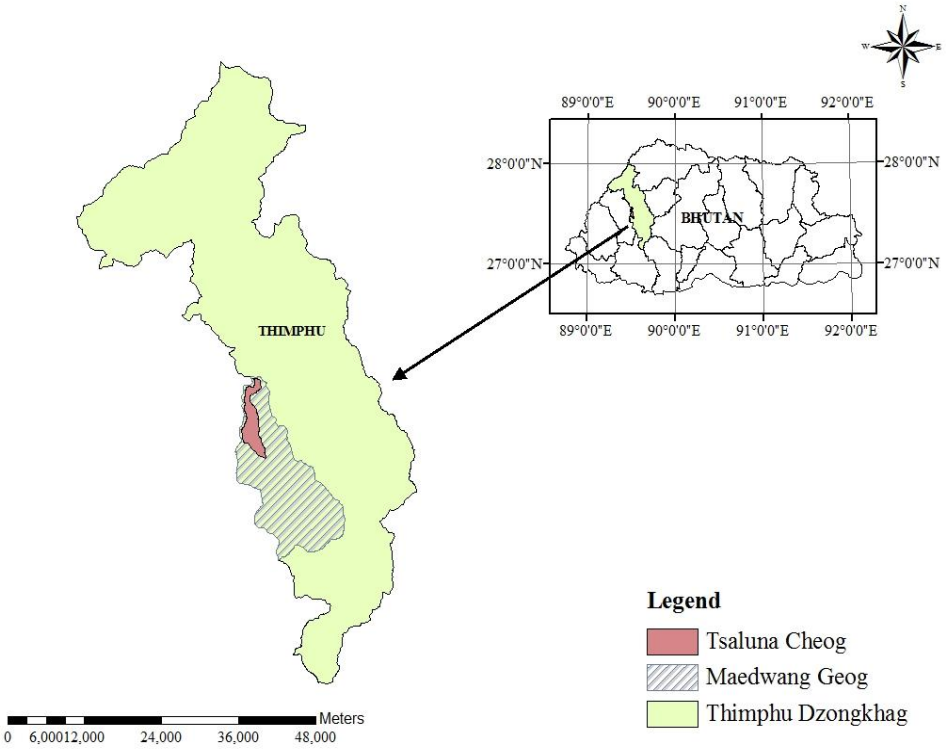

Figure 1: Map and location of study area.

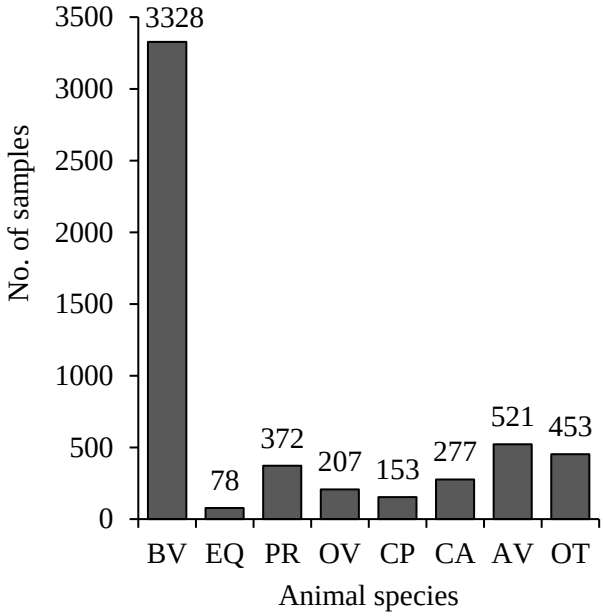

Figure 2: Prevalence of endo-parasites.

2.4 Preparation of plant suspension

The dry wormwood leaves were collected in the month of December, 2014 from Jhazam area under Thimphu. According to National Institute of Traditional Medicine (NITM), the abundantly grown species of Artemisia is *Vulgaris* species. The ripped papaya was collected from Gelephu and Phuntsholing. The seeds were extracted from papaya pods and washed with tap water and sun dried for 3 days until seeds have dried completely. The wormwood leaves and papaya seeds were pulverized in an electric grinder for 15 minutes and sieved for the final powder. The powder was weighed to 200 g and blended with 500 ml of warm water. The suspension was kept overnight and then administered in the next day. After treatment, the fecal samples were collected for egg counts. Mustard oil was purchased directly from the market and administered at 200 ml per treatment to large animal and around 100 ml to young cattle. A total of 104 fecal samples were collected and analyzed.

2.5 Data analysis

Primary data were collected by using semi-structured questionnaire. Graphs were prepared in Microsoft Excel. An analysis of variance and a friedman's test (mean and standard deviation) were conducted to determine reduction in endo-parasitic load under three different treatments. Pairwise comparisons were made to detect significant differences between treatments. The dataset was analyzed with SPSS software version 16.0.

3. RESULTS

3.1 Composition of infection

About 65% samples tested positive to endo-parasites comprising 39.42% *Ascaris*, 11.53% *Coccidia*, 2.88% *Strongyles* and 0.96% *fasciola* (Figure 2). About 10.57% were mixed infection and 34.62% tested negative.

3.2 Effects of plant suspension on endo-parasites

The effects of plant suspension on endo-parasites at different intervals are presented in Table 1. For the wormwood treatment, the initial mean egg count was 8200 eggs per gram (epg) and after treatment, the count was 5100, 2100, 1400 epg on week one, two and three, respectively. The mean egg count was reduced by 83% in three weeks. There was a significant difference in worm load (epg counts) among four treatment intervals.

For the papaya seed treatment, before treatment, the worm count was 8700 and the worm counts were 2300, 2800 and 1700 epg on week one, two and three, respectively. There was a significant difference in worm load (egg counts) between treatment intervals. This indicates that papaya seeds have de-worming properties and could control endo-parasites such as *Ascaris* and *Coccidia*. However, there was no statistical difference between first, second and third treatment, which means repeated dose after first treatment has no concurrent effect unlike wormwood leaves suspension.

Under treatment with mustard oil, the worm count was 6400 epg before treatment, and in the entire treatment course, the worm count in week one was 2400 epg, week two 1700 epg and week three 900 epg. The significant difference was observed in epg count with mustard oil treatment at different intervals. The results obtained before and after treatment (one week, two weeks and three weeks interval) were significantly different. This indicates that mustard oil does have anthelmintic effects. However, repeated treatments did not show much efficacy as compared to other two treatments.

4. DISCUSSION

Out of 104 samples analyzed, the test results show high prevalence of parasites in the study area. This agrees with the finding of National Center for Animal Health (NCAH 2013). The study area has open grazing land, which seems to have been contaminated by parasite. The infected cattle also appear to have developed resistance due to frequent usage of drugs. Resistance of internal parasite of livestock to drugs is reported worldwide (Waller et al. 2001).

Most wormwood species have de-worming properties (Laudato and Capass 2013). Junquera (2014) reported that the whole plant of wormwood has constituents of "Thujone", which is used against

Table 1: Mean epg count under three plants suspension treatments at different intervals (Mean ± SD). Means with different letters within a row differ significantly at p≤0.05.

Treatment	Count				Reduction in final worm count (%)
	Before treatment	1 week after treatment	2 weeks after treatment	3 weeks after treatment	
Wormwood plant extract	820±399 a	510±292 b	210±129 c	140±70 d	83.0
Papaya plant extract	870±523 a	230±221 b	280±235 b	170±82 b	80.0
Mustard plant oil	640±532 a	240±375 b	170±125 b	90±32 b	86.0

parasitic round worms (e.g. Haemonchus, Bunostomum and Protostrongylus). Repeated dose of plant extract at different intervals helped in eliminating worms. This could be due to consistent use of oil in the stipulated time intervals. Debairacli (1973) reported that the infusion of dry flower of wormwood is noxious for Bunostomum, Dictyocaulus and Protostrongylus. Lans et al. (2007) confirmed that the de-worming of pets and pigs with *A. absinthium, A. annua, A. cina* and *A. vulgaris* was effective against *Ascaris*. Elgarhy and Mahmoud (2002) also found that wormwood seeds effectively reduced the egg count of *Strongyloides*.

Repeated treatment with papaya seed suspension showed no concurrent effects on parasites. This might be due to less concentrations of plant extract resulting in less effect on worms. It contradicts the finding of Satrija et al. (1994) who reported that the use of water extracts of papaya seeds decreases *Ascaridia galli* infections in poultry. The papain in papaya seed has been reported to reduce worms (Ameen et al. 2010). The main anthelmintic property in papaya is benzyl isothiocyanate that destroys worms (Krishna et al. 2008). In a study conducted by Ameen et al. (2010), the powder and aqueous solution treatment significantly reduced worm burden in sheep.

Mustard oil proved effective during initial treatments but after repeated treatment it became less effective. This may be due to less dosage (200ml) per adult cattle. The oil has anthelmintic ingredients, which was reported to cause death of earthworms (Manthri et al. 2001; Manthri et al. 2011).

5. CONCLUSIONS

Plants extracts and oil tested in this study proved to be effective in controlling worms. Similar study needs to be carried out to test the effectiveness of other vegetable extracts of pumpkin seed, ginger, garlic, marigold flower and onion. In this study, the plant suspensions were prepared with water. However, the use of these plants with other medium and extracts should be explored in future studies. This study tested plant extracts in cattle only. There is a need to extend such studies to other animal species such as equine, swine and canine.

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