Bhutan Journal of Animal Science (BJAS)
Volume 4, Issue 1, Page 47-53, 2020

Full length paper

STAPHANOFILARIAL DERMATITIS (HUMPSORE) TREATMENT TRIAL IN TASHICHOLING, SAMTSE DISTRICT

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ABSTRACT: Humpsore is a skin disease in cattle caused by a vector borne nematode belonging to the genus, *Staphanofilaria*. To select a treatment option with the highest sore recovery rate, a treatment trial was conducted in four villages under Tashicholing Drungkhag, Samtse involving 80 humpsore affected animals. These animals were randomly assigned to four groups namely: 1) group 1 (single ivermectin injection sub-cutaneously @ 1ml/50 kg body weight + topical application of Zinc Oxide (ZnO) ointment daily); 2) group 2 (single levamisole HCl injection sub-cutaneously @ 1ml/50 kg body weightt + topical application of Zinc Oxide (ZnO) ointment daily); 3) group 3 (Topical application of Zinc Oxide (ZnO) ointment daily); and group 4 (untreated control). ZnO ointment was applied daily for the entire trial period. Ivermectin and Levamisole injection was given on the second day of the trial. The sore size (in cm) was measured before application of any treatment and thereafter on weekly interval. The change in mean sore size for each group was computed using a one sample t-test while the comparison between group was made using one-way ANOVA and pair-wise t-test. In total 24 deep lesion scrapings were collected from the animals that were enrolled in the trial as well as from those which were not. Samples were processed using conventional sedimentation method and the Baermann' technique for recovery of the parasite. By the end of the trial, complete healing of the lesion was observed in 14 (70%) animals under group 1, 12 (60%) under group 2 and 13 (62%) under group 3. Analysis showed that the reduction in the mean sore size for all the group was statistically significant (p <0.0001) except for the control group (p=0.06). There was no statistically significant difference in the mean sore size between the groups before the start of the trial (p=0.24). By the third week, the difference in the mean sore size for all the treatment groups, group 1 (p <0.0001), group 2 (p <0.001) and group three (p <0.0001) was statistically significant difference from the control group. Microfilaria was isolated in 9 of the 24 samples with a microfilaria recovery rate of 37.5%. This study has further validated that ivermectin injection @ 1 ml/50kg body weight with topical application of zinc oxide ointment for three weeks has the highest efficacy against humpsore. It also proposes a speculation, for further validation, that isolating active microfilaria is more likely in samples that are collected in the later part of the day.

Keywords: Bhutan; humpsore; microfilaria; parasitic dermatitis; *Staphanofilaria assamensis.*

1. INTRODUCTION

Staphanofilarial dermatitis, commonly called as humpsore, is a vector borne skin disease caused by a nematode parasite belonging to the genus *Stephanofilaria*. The muscid flies are known to be the common biological vector for the parasite (Saparov et al. 2014). Based on the species of parasite affecting their

host, the lesion can be found in different parts of the body. For instance, S. *assamensis* is known to cause a condition called humpsore as the lesions in animals are commonly seen on and around the hump. While the *S. stilisi* usually affects the ventral line of the host's body. The lesions are characterized by alopecia and ulcerative nodular dermatitis in cattle. Humpsore is mostly prevalent in the Indian sub-continent and south-east Asian countries (Rai et al. 1994). The impact of this disease is numerous. Besides compromising the animal welfare, economic loses result from the lose of hide value, draught power, decreased milk production, and decreased growth rate are reported (Rai et al. 2010). In Bhutan, humpsore is highly prevalent in the southern foothills of the country with hot and humid weather conditions that favour biting fly activities. In a study conducted at Sarpang Dzongkhag, Dukpa et al. (2008) reported humpsore herd prevalence of 36.3%. Treating humpsore is difficult as it requires a multifactorial approach targeted towards management of the parasite, biological vector, and environment. Approaches aligned towards any one of these has yielded no success, and if at all, just for a transient period. As early as 1948, researchers have conducted several treatment trials using different parasiticides such as acriflavine, anthiomalin, anthisan, antimony tartarate, fenitrothin formula, florocid, formalin, levamisole, methyridine, promintic, sodium arsenite, suramin, tartar emetic, tetramisole, tobacco extract, vaseline, neem leave extract and reported varying efficacy (Rai et al. 1994; Dukpa et al. 2008; Al Masud et al. 2017; Johnson 1989; Ibrahim et al. 2013; Singh et al. 2014). In a maiden study conducted in Bhutan, Dukpa et al. (2008) reported that the combination of ivermectin injection and zinc oxide ointment showed the highest treatment efficacy rate of 85% followed by the group comprising of the combination of levamisole and zinc oxide application (80%), Salycilic acid and butox ointment (75%) and Coumaphous ointment (70%). However, the trial involved only 20 animals. There are no trials conducted thus far in other humpsore endemic parts of the country. Humpsore has remained a persistent problem in the southern parts of the country inflicting heavy economic loses to the marginal farmers and compromising animal welfare. Therefore, this trial was conducted to identify and document the most effective treatment option against humpsore and their associated benefit in veterinary medicine indenting and supply at the field level.

2. MATERIALS AND METHODS

2.1 Study area

Of the two southern districts, Chukha and Samtse, under the western region of Bhutan, Samtse reports the highest number of humpsore cases. Therefore, Samtse was selected for the trial. Under Samtse dzongkhag, several sub-districts report humpsore in cattle. However, based on the convenience (logistic), Tashicholing was selected. Humpsore affected cattle from four villages namely, Dewachen, Peljorling A, Peljorling B and Norgangsa under Tashicholing geogs were enrolled. Tashicholing is a sub-tropical sub-district. The annual average maximum temperature during 2017 was 29.1 degrees centigrade and the annual average minimum temperature was 18.3 degrees centigrade. The annual total rainfall received was 5763.2 mm that year.

2.2 Study design

Before the start of the trial, two days meeting was arranged with the dairy farmers who owned cattle that were affected with humpsore from the selected study areas. This meeting was organized to sensitize them about the study and obtain their consent to participate, besides educating them on the pathogenesis, treatment options and prevention of humpsore in cattle. The meeting also provided us an opportunity to learn about the ethnoveterinary practices that are currently used to treat humpsore.

Figure 1: Painting the horn of the animals selected for the trial: A) red colour horn corresponds to the treatment group 1, B) Blue colour corresponds to the group 2, C) Green colour corresponds to the group 3 and D) Brown colour to untreated control

After the meeting, with the help of the gewog livestock extension agent, households in the villages mentioned above were visited. Based on the convenience, households nearby the livestock extension centre were visited first and then the visit stretched out until the required number of humpsore affected animals was met. In this study, all the animals that had ulcerative nodular lesion on their skin were considered humpsore positive. As the team visited the humpsore affected cattle, best effort was made to randomly assign animals to the treatment groups designed for the study. In total 80 humpsore affected animals were enrolled for the trial, 20 each in the group 1 & 2 and 21 and 19 each in group three and four respectively. And for identification, apart from taking the photographs of each animal, the horns were

painted corresponding to the treatment that they would receive. The animals that were assigned to group 1, 2 and 3 were painted red, blue, and green respectively while the untreated controls were painted brown (Figure 1). For the animals that were dehorned, their hooves were painted. The paints although faded over the trial period, it was clear enough to recognize till the end of the trial.

2.3 Data collection

A record of the animals such as age, gender, breed, weight and the size, number and location of the sore was collected during the inventory. Weight of the animals was estimated using a body weight estimating tape. For the purpose of this study, the longest open wound was recorded as the sore size. The sore size was measured using a plastic ruler and recorded in centimeters.

2.4 Treatment protocols

Three treatment options were designed for this study (Figure 2). The first group received a single injection of ivermectin sub-cutaneously @1ml/50kg body weight and application of Zinc Oxide (ZnO) ointment daily while the second received a single shot of levamisole hydrochloride sub-cutaneously @7mg per kg body weight and application of ZnO ointment once daily. The animals in the third group was applied only ZnO ointment daily. Depending on the size of the sore, around 15-30 gm of ZnO was applied once daily (between 6 am to 9 am) on the lesion for successive 21 days. The animals in group 4 (untreated control) was not provided any treatment.

A clear instruction was reiterated to the animal owners in the control group to ensure that no treatment, in any form, must be given to the animals until the completion of the trial. Starting 9th day, cypermethrin (5%) was sprayed around the wound to control fly activity for group 1, 2 and 3.

The presentation of the ZnO used was 15% W/W in white soft paraffin jelly. The ointment was prepared at the Livestock Extension Center. A required amount of ZnO powder was taken on an ointment slab and mixed. Subsequently, a required amount of white soft paraffin jelly was weighed. Then the ZnO powder and paraffin jelly was uniformly mixed to form a homogenous ointment. The preparation was stored in small containers which were carried to the field next day for application.

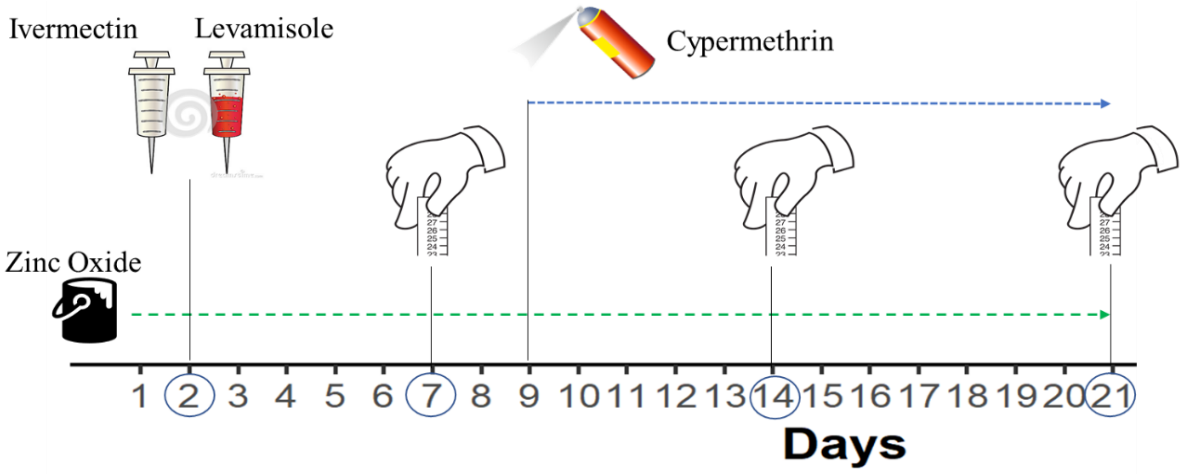

Figure 2: Trail time line and different activities undertaken once the trial was initiated

2.5 Efficacy indicator

To assess the effect of treatment, the sore size was measured at a weekly interval. As an attempt to control measurement bias, the study recorded the cattle owners' subjective assessment of treatment on four qualitative scales-no improvement, mild improvement, drastic improvement and complete healing- on the first and second week after the start of the trial. In this study, the reduction in the size of the sore was taken as an efficacy indicator. In animals having more than one sore, the size of the largest sore was recorded, and used for analysis.

2.6 Sample collection and isolation of microfilaria

Deep skin scraping samples were randomly collected from 24 humpsore affected animals. Some of the animals were those enrolled in the study while some were not. After incubating the samples in normal saline for 2 hours, the samples were centrifuged at 1000 revolutions per minute (rpm) for 3 minutes. For isolation of adult parasite, the tissue debris were separated by decantation and presence of actively motile adults were examined as described by Singh et al. (8). An aliquot of tissue debris, after thorough mixing, were placed on a glass slide and examined for the presence of microfilaria under microscope (10 and 40X). The study also adopted Baermann's technique to overcome the limitation

(obscuring field) of observing tissue debris under the microscope. Skin scrapping collected from 5-6 animals were pooled in a muslin cloth which was then suspended in an inverted funnel containing a distilled water. It was ensured that the sample remained fully immersed in the water. The sample was incubated for 24 hours. After incubation, the filtrate was taken in a centrifuge pipette and centrifuged at 1000 rpm for 3 minutes. Discarding the supernatant, the sediment was observed for the presence of microfilaria. Due to the lack of adequate facilities, identification of isolated parasites was not carried out.

2.7 Data analysis

The sore size of each animal for three measurements were managed in Microsoft excel 2013 (Microsoft excel 2013, Redmond, USA). All the analyses were conducted in R statistical software using inbuilt packages "dplyr", "descr" and "ggplot2". For analysis, measurement of the largest sore was used in those animals that had more than one sore. Descriptive analysis was conducted to derive the maximum, minimum and mean size of the sore for all the treatment groups. To assess the effect of each treatment on the reduction of the sore size, the mean sore size of each treatment group before and weekly (first week, second week and third week) after the start of the trial was compared on weekly interval. The comparison of the mean sore size of each treatment group before and after the start of trial was made using a one sample t-test. The comparison of the mean sore size between the treatment groups before and after the start of the trial was conducted using one-way analysis of variance (ANOVA) and pairwise t-test. All the test with p-value of less than 0.05 was considered statistically significant.

3. RESULTS

In total, 80 humpsore affected cattle were included in the trial of which 62 were female and 18 males. Forty-five cattle were jersey cross while the rest were local cattle. Except for four heifers, rest of the affected animals were all adults. The youngest animal affected was the 11 months old heifer. The sore was observed mainly on the hump region, neck, forehead, base of the ear, inner eye canthus. Forty-four animals had humpsore lesion only on the single location while the rest had on multiple locations.

3.1 Mean sore size before treatment and at weekly interval categorized by group

The mean sore size has decreased in all the treatment groups including the control group. The details of the decreasing sore size are provided in Table 1 and graphically presented in figure 6. The overall mean sore size before the treatment started was 7.8 cm (range=2-17cm). One week after the start of treatment, the overall mean sore size decreased to 5.5 cm (range=0-15cm) and subsequently to 2.8 cm (0-9.5cm) and 1.8 cm (0-10cm) second and third week respectively.

Table 1: Details of the sore size at weekly interval for the different treatment groups

Treatment group	Mean sore size (in cm)			
	Before trial	**First week**	**Second week**	**Third week**
The overall	7.8 (2-17)	5.5 (0-15)	2.8 (0-9.5)	1.8 (0-10)
Group 1	9.9 (5-17)	6.6 (0-15)	2.5 (0-9.5)	0.67 (0-5)
Group 2	8.05 (3.5-15)	4.8 (0-15)	2.4 (0-9)	1.5 (0-10)
Group 3	7.7 (4-15)	6.0 (2-14)	2.2 (0-6)	1.1 (0-5.5)
Control	5.7 (2-15)	4.6 (2-14)	4.3 (0-9)	4.1 (0-10)

The figures in the parenthesis represent the range of sore size

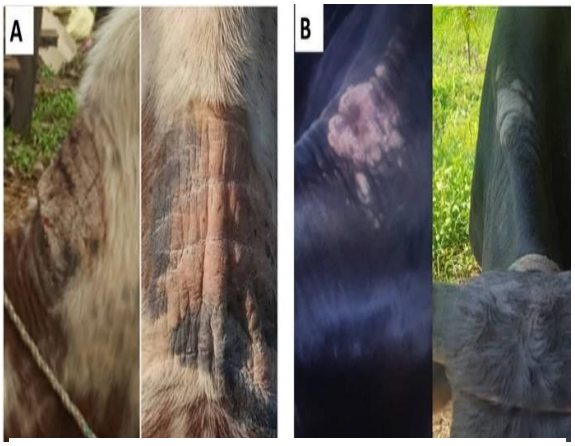

Figure 4: Image showing the humpsore before and after treatment trial

The paired t-test analysis showed that the reduction in the mean sore size for all the treatment group before the start of the trial and after completion of the trial was statistically significant (p <0.0001) except for the control group (p=0.06). Complete healing of the lesion was observed in 14 (70%) animals under the group 1, 12 (60%) under group 2 and 13 (62%) under group 3. Surprisingly, complete healing of humpsore lesion was also observed in 2 (10%) animals under the control group. There was no statistically significant difference in the mean sore size between the groups before the start of the

trial (p=0.24). By the third week, the difference in the mean sore size for all the treatment groups, group 1 (p<0.0001), group 2 (p <0.001) and group three (p <0.0001) was statistically significant from the control group. Whereas, there was no difference in the mean sore size between the groups where treatment was given.

3.2 Subjective assessment

The farmers' subjective assessment of the lesion healing supported the assessment in this study. One week after the trial, 16 farmers under group 1 reported drastic improvement in the humpsore lesion while 4 reported mild improvement. Similarly, for the group 2, 1 farmer reported complete healing, while 16 reported drastic improvement and 3 mild. For group three, 15 farmers reported drastic improvement while 5 reported a mild improvement and 1 reported no improvement in the lesion.

Two weeks after the trial, for the group 1, 3 farmers reported complete healing while the rest reported drastic improvement (Figure 4). For the group two, 4 farmers reported complete healing while the rest drastic improvement. For group 3, 18 famers reported drastic improvement, 2 reported mild improvement and one reported no improvement in the lesion.

3.3 Recovery of parasite

This study could not isolate adult parasite in any of the sample. However, microfilaria was isolated in 9 of the 24 samples with a microfilaria recovery rate of 37.5% (Figure 5). The active microfilaria was mostly observed in the samples that were collected in the evening and examined later that day as opposed to the samples that were collected in the morning and examined during the mid-day. Interestingly, the study could have also isolated microfilaria using Baermann's technique. However, since the samples were incubated for 24 hours, live microfilaria could not be isolated using this technique. The microfilaria thus isolated presented in an open circle or c-shape form.

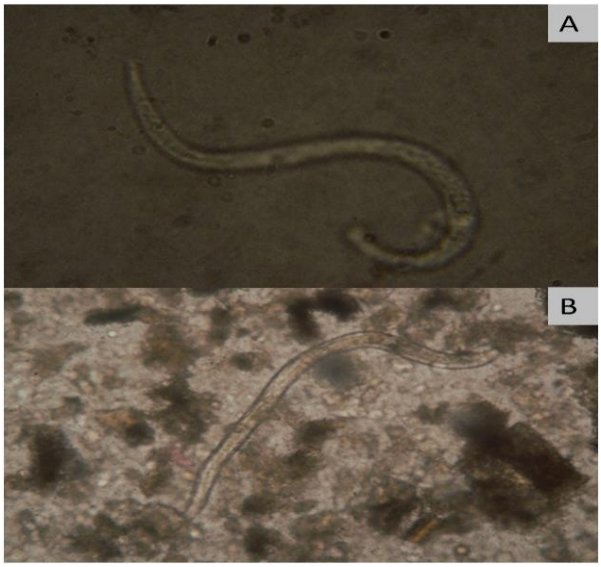

Figure 3: Microfilaria observed under oil immersion isolated using Baermann's technique(A) and observed under 40X using direct method (B)

4. DISCUSSION

The trial compared three commonly used treatment options for treating humpsore. All the three treatment

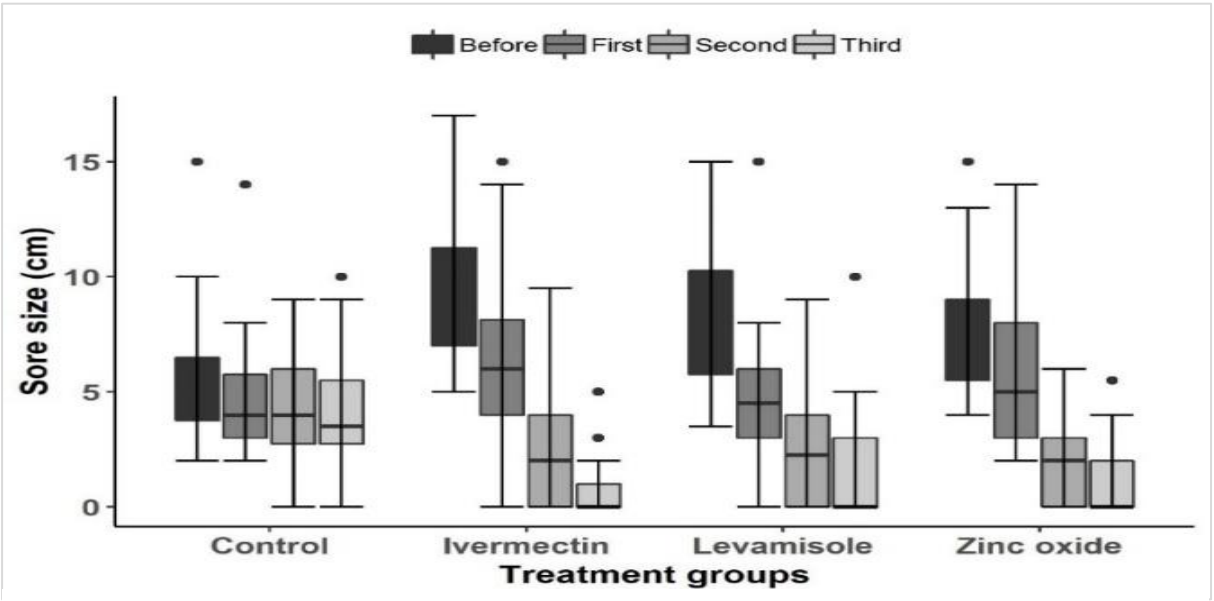

Figure 4: Figures showing the variation in the sore size on a weekly interval for different treatment groups

options brought about drastic improvement in humpsore lesion. Group 1 (Ivermectin and daily application of zinc oxide ointment) had the highest cure rate (70%). This finding agrees with the finding reported by Dukpa et al. (2008) and Johnson (1989). Contrast to the finding reported by Dukpa et al. (2008), group three had efficacy rate of 62% followed by group two (60%). The finding was also in contrast with the study conducted by Rai *et al*. (1994) who reported that application of zinc oxide ointment alone failed to cure a single case while combination of levamisole HCl and topical zinc ointment application cured all the animals. Taking into the account of cattle owners' assessment, the cure rate after second week of the trial was higher in the group 2 than other groups; however, it was observed that a common side effect associated to levamisole HCl injection was swelling at the injection site.

In this study, humpsore was largely restricted to adult animals. This could be attributed to the differences in the way adult and young cattle are managed. A predisposing factor to humpsore development is a break in epidermis (wound) where biological vectors can deposit the microfilaria. Unlike the calves, which are mostly tethered and managed in the cattle shed, adult cattle are free grazed in the forest whereby the chances of sustaining scratch wounds are higher.

Apart from the humpsore treatment services that the farmers avail from the nearest livestock extension centre, they reported resorting to ethnoveterinary practices such as application of used engine oil, turmeric powder mixed in mustard oil, application of soot, application of pork lard and battery contents. The efficacy of these agents in curing humpsore is still unknown. The study suspects that the 10% cure rate in the untreated control group could have resulted from farmers non-compliance to the instructions of not using any medication in the control animals till the end of the trial.

Except for topical application of Zinc Oxide alone, which do not have anti-microfilarial property, two other treatment groups were designed to kill microfilaria in the lesion (ivermectin and levamisole HCl), prevent biological vectors from further depositing microfilaria (Zinc oxide ointment paste and cypermethrin spray around the lesion), and expediting wound healing (Zinc Oxide ointment). However, finding group 3 (Zinc Oxide alone) having more cure rate than group 2 (levamisole HCl and topical application of Zinc Oxide ointment) was surprising.

No adult parasites could be isolated. This could be because of the issues associated with sample collection. Some of the earlier studies have reported not being able to isolate adult parasites from the lesion (Dukpa et al. 2008) while those who could were mostly in a tissue section (Saparov et al 2014; Md. Nazur Islam et al. 2018). Thus, this finding points out to variation in the location between adult parasites and microfilaria. In contrast, microfilaria was recovered in 38% of the samples that were collected. Our observation of detecting active microfilaria in the samples collected at the later part of the day and observed subsequently than in sample collected during the earlier part of the day could be associated with the biological rhythm of the microfilaria. Although the number of samples collected and examined were limited, it was speculated that the *Staphanofilaria* microfilaria recovery would be enhanced in the samples collected in the later part of the day and examined subsequently. However, this claim requires further validation. One problem associated with direct microscopic examination of tissue sediment was the risk of missing dead microfilaria. The tissue and hair particle made the microscopic field crowded obscuring the observation field, and thus decreasing the sensitivity of this method of examination. The Baermann's technique proved to be very effective in isolating microfilaria in addition to the advantage of having no crowded microscopic field as the direct method. The Baermann's technique was employed for the first time in isolating *Staphanofilaria* microfilaria through this study. While the study could only observe dead microfilaria, it was assumed that the incubating time can be calibrated (shortened) to isolate live microfilaria using this technique.

While many cattle have been affected by *Staphanofilaria spp.*, it was surprising to see minimal number of effective drugs, such as ivermectin and levamisole, available in the livestock centres. For instance, the total supply of ivermectin to Tashicholing geog for the fiscal year 2018-2019 was about 10% of the total ivermectin that would have been required. This observation underlines the gaps in the current veterinary drug indenting and supply mechanism. If the indenting of veterinary medicine is done based on the need relating to the types of cases common in the area followed by supply of medicines as per the indent, the current problem of drug shortages and the disparity in the indent and supply of the drugs can be drastically alleviated.

This trial has some limitations. Firstly, as described in the materials and methods section, due to the withdrawal period for the ivermectin and levamisole HCl in milk, it was ensured that milking animals were not placed in group 1 and group 2 where ivermectin and levamisole respectively, was used. This must have resulted in the selection bias. Secondly, for the objective assessment of the lesion recovery by the measuring the sore size, the study could have blinded the assessment using officials who were not involved in the trial. However, due to the shortage in human resources, the individuals involved in the trial were involved in measuring the sore size. Furthermore, not the same person measured the sore sizes. Differences in the measurement resulting from different assessors must have resulted and this was evident from some of the sore sizes observed in this study. Due to the limited trial period and difficulty in

accurately assessing the humpsore based on isolation of etiological agent, the study assumed all the animals having sore on the skin as humpsore positive animals. This assumption can be questioned as numerous other agents can be attributed to skin lesion, particularly dermatophytes which produces similar lesion in animals. If conducted in future, it would bring improvement in selection of case animals (based on isolation of etiological agent), randomization and allocation to treatment groups, blinding while assessing/measuring the sore size and use proper methods to identify the isolated microfilaria.

5. CONCLUSION

This study has further validated that ivermectin injection @ 1 ml/50kg body weight with topical application of zincoxide ointment for three weeks has the highest efficacy against humpsore. Furthermore, the use of levamisole HCl in place of ivermectin had a comparable efficacy. Although topical application of Zinc Oxide alone showed a comparable efficacy in treating humpsore, as ZnO doesn't have anti-microfilarial activity, adopting to this option as a choice of treatment may be made with caution. The study also proposes a speculation, for further validation, that isolating active microfilaria is more likely in samples that are collected in the later part of the day

ACKNOWLEDGEMENT

The team would like to thank Dasho Director General for his continued guidance and directives. We would extend our acknowledgement to the Dzongkhag Livestock Sector, Samtse for facilitating this study and providing support where required. Further, our heartfelt thanks also go to the in-charge RNR-EC Tashicholing for his support during the entire study period. We also would like to thank our support staff during the field work. Finally, we extend our special thanks to the cattle owners of Tashicholing and those animals that were involved in the trial. Mr. Yab Raj Ghalley and Ms. Januki Chettri for their effort and tireless commitment in supporting the team.

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