



## Comparative efficacy of tobacco leaves ointment and neem leaves extract against stephanofilariasis in cattle

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### ABSTRACT

Stephanofilariasis is one of the economically important parasitic diseases caused by *Stephanofilaria assamensis* and is endemic to Bangladesh. Here, we describe the prevalence of the disease and development of suitable measures to control the disease. We found that 20% animals were affected with humpsore, and the prevalence was significantly higher in older animals. Sex of the animals was an important determinant and the disease was more common in male. Prevalence of the worm was significantly higher in indigenous cattle. Use of the animal also influenced the prevalence and the disease was more common in draft-animals. Our blind, *in vivo* trial revealed that 15% tobacco ointment (15 g tobacco powder mixed with 85 g vaseline) caused complete healing of sores in all treated animals within 28 days whereas neem leaves extract failed to induce complete healing. Taken together, the results suggest that humpsore is still endemic in Bangladesh and is a big problem in profitable cattle rearing, and 15% tobacco ointment can be successfully used to treat the disease.

### INTRODUCTION

Stephanofilariasis is a chronic, parasitic dermatitis caused by the worm *Stephanofilaria assamensis* (Nematoda: Filaridae), which is characterized by intensive itching, rubbing of the injured area with hard object, dry and rough skin, and alopecia (Soulsby, 1982, Miyakawa et al, 2008). Stephanofilariasis is commonly known as humpsore because the lesion is usually developed on or near the hump and neck regions of cattle. However, lesion may also be developed in the other parts of the body and even in the inner canthus of the eye and scrotum (Watrelet-Virieux and Pin, 2006). This disease is transmitted by the vector *Musca conducens* and distributed throughout the world (Baki and Dewan, 1975, Phukut et al. 2005, Rai et al. 2010, de Novaes and Miyashita, 2007). Stephanofilariasis is endemic in Bangladesh and India (Baki and Dewan, 1975,

Phukut et al., 2005, Rai et al, 2010), except in hilly region, causing severe economic losses due to loss of productivity, and decreased value of the animals and their skin as well. They can affect buffalos, goats, elephants, black rhinoceros and nilgai. Infection in humans with the worm has also been reported (de Novaes and Miyashita, 2007). Secondary bacterial infection and/or maggot infestation aggravate the situation. Besides, stephanofilariasis cause lather defects (Baki and Dewan, 1975) and probably can cause an annual economic loss of US\$ 220.95 million in Bangladesh.

Indiscriminate use of anthelmintics for the treatment and control of helminth diseases in animals has led to the development of anthelmintic resistance (Peter et al., 2008). Different countries like Indonesia, India, and Thailand produce a variety of medical products

such as anthelmintic, anti-diarrheal preparation, astringents, skin ointments and clearing agents from indigenous plants and herbs, which are farmers friendly, cost-effective and environmentally friendly (Rao et al, 1986, Rahman et al., 2009). A larger number of plants are available in Bangladesh, which have specific or broad spectrum anthelmintic activities (Sujon et al., 2008) and are used to combat parasitic infection in livestock. In India, in ayurveda and unani system of medicine, neem seeds, fruits, leaves, oil, roots and bark are used. Neem products act against various types of nematodes. Certain limnoid fractions extracted from Neem kernels are effective against root knot nematodes (Singh and Patel, 2015), the most devastating nematodes affecting plants. It is also a common remedy against maggots. In Bangladesh and India, villagers use neem oil to kill lice and other ectoparasites (Al-Quraishy et al., 2012, Mehlhorn et al., 2011). On the other hand, tobacco leaves (*Nicotiana tabacum*) contains an alkaloid, nicotine, which is water soluble and it has an anti parasitic action.

To treat humpsore, usually ointment containing organophosphorus or organocarbamate is used ((Baki and Dewan, 1975) that are potentially hazardous for animals and humans. In addition to acute toxic effects, these chemicals have both residual and cumulative effects. On the other hand, ivermectin, a broad spectrum anthelmintic, can kill the worm efficiently (Brammer and Gibson, 1996) but is not effective against bacterial superinfection, and also does not work as repellent against the vector fly. Therefore, we studied here the comparative efficacy of tobacco leaves ointment and neem leaves extract against hump sore in cattle.

## MATERIALS AND METHODS

### Animals and experimental design

A total of 75 cattle of both sexes of different ages were examined during the period of June to November/ 2013 to study the prevalence of humpsore. The site of the lesion as well as age, sex, breed and use of animals were recorded. A total of 15 clinical cases of stephanofilial dermatitis having typical humpsore lesion on

hump and neck region were selected to study the efficacy of selected plants. The lesions of humpsore were 3.5-10.5 cm in diameter. These 15 cattle were divided into 3 groups each consisting of 05 cattle; group A was treated with tobacco leaves, group B with neem leaves extract, and group C was kept as untreated control. Farmers were advised to provide almost similar feed and water *ad libitum*.

### Preparation of plant products

Neem leaves were cut into small pieces and mashed with the help of mortar and pestles. To prepare 15% aqueous extract, 15 g of paste was thoroughly suspended in a total volume of 100 ml of distilled water. The suspension was kept overnight at 40 °C and filtered. On the other hand, fine powder was prepared with 15 g of dried tobacco leaves.

### Application of drugs

Tobacco ointment (15 g tobacco powder mixed with 85 g Vaseline) was applied topically twice daily to the lesion of the cattle of group-A and 15% neem extract were used topically in affected area of cattle of group B. Efficacy was assessed by two blind investigators up to 28 days.

### General effect of treatment

The body weights of animals were recorded at day0 and day 30 post treatment (PT). To measure toxic effects, blood samples were collected from at day 0 and day 28, and total erythrocyte count (TEC), hemoglobin (Hb), packed cell volume (PCV) and erythrocyte sedimentation rate (ESR) were estimated.

## RESULTS AND DISCUSSION

### Prevalence of stephanofilariasis (humpsore) in cattle

To determine the prevalence of humpsore at Dinajpur district a total of 75 cattle were examined. Out of 75 cattle 15 (20%) were found affected with stephanofilariasis, indicating that the disease is endemic to the study area of Bangladesh. The present finding is almost similar

to the earlier observation of Nooruddin and Dey (1990) who recorded 14.60% prevalence of humpsore in cattle in Bangladesh. The disease was commonly seen in the hump and neck regions. In this study, it is found that males were more susceptible to the infection and conforms to the findings of Johnson et al. (1986). However, Akter et al. (2016) reported that females are more prone to the disease. We observed that indigenous cattle used in draft purpose were more susceptible to the disease, suggesting breed, sex and use of animals are dominant risk factors, supported by the findings of Johnson and Toleman (1988).

### Comparative efficacy of tobacco ointment and neem leaves extract

To determine efficacy of tobacco leaves, cattle of group A were treated with tobacco ointment topically twice daily that showed gradual improvement of the lesions within day14 PT, and marked improvement of lesions was observed within day21, which was characterized by the reduction of irritation and size of the sore. After day28 PT the lesions were completely healed up with the formation of scar tissue (Table 1). Tobacco leaves contain nicotine, which is water soluble and it has a strong anti-parasitic action.

Table 1

Effect of tobacco ointment and neem leaves extract against humpsore in cattle.

Days	Sore diameter, cm (Reduction, %)		
	Group A (%)	Group B (%)	Group C (%)
Day0	5.9±0.14 <sup>a</sup> (0)	6.24±0.16 <sup>a</sup> (0)	6.4±.31 (0)
Day14	2.3±0.11 <sup>b</sup> (61)	3.60±0.25 <sup>b</sup> (43.21)	6.6±.36 (0)
Day21	0.7±0.01 <sup>b</sup> (88.14)	2.25±0.07 <sup>b</sup> (64.52)	6.8±.39 (0)
Day28	0 <sup>ab</sup> (100)	1.05±0.05 <sup>ab</sup> (83.03)	7.0±.41 (0)

Data have been presented here as mean±SE, and values with different superscript in the same column are statistically significant (p<0.05)

On the other hand, cattle belonging to the group B were treated with neem leaves and assessed. Within 2-7<sup>th</sup> day there was significant

improvement, characterized by the reduction of the size of lesions (Table 1). Sujan et al. 2008 also found that the efficacy of neem leaf was 81% at day 21 in cattle. However, complete recovery was not observed in neem extract treated group even after days 28 PT (Table 1), suggesting higher efficacy of 15% tobacco ointment. Neem contains several active ingredients and certain limnoid fractions extracted from neem kernels are active against root knot nematodes (Singh and Patel 2015). Lower activity of neem leaves extract may be due to different formulation. Tobacco was applied as an ointment that covered the wound for longer time. On the other hand, neem extract was used an aqueous extract without any vehicle that dried up easily.

### General effect of treatment

The body weight of treated cattle (Group A & B) was increased to the extent of 3.3% and 2.17% on the day 28, following tobacco and neem leaves extract application, respectively. On the other hand, the body weight of control group decreased gradually (Table 2). Due to the treatment lesions healed up rapidly. Animals became calm and their food intake increased resulting improvement of body condition. No adverse effects were observed on hematological parameters of treated cattle by any of the formulation (neem leaves extract and tobacco ointment) used in this experiment (Table 3), suggesting both of the plant formulation are safe for animals.

Table 2

Effect of tobacco ointment and neem leaves extract on body weight in cattle.

Group	Body weight (kg)		Gain, kg (%)
	Day0	Day28	
A	125.2±7.5	128.5±9.5	3.3(2.6) <sup>a</sup>
B	124.4±9.3	127.1±3.4	2.7(2.17) <sup>a</sup>
C	126.7±5.5	124.6±2.6	-2.1 (0) <sup>b</sup>

Data have been presented here as mean±SE, and values with different superscript in the same column are statistically significant (p<0.05)

Collectively, our results suggest that humpsore is still endemic to the country and 15% tobacco ointment can safely be used for the treatment of the disease and to control the worm.

Table 3  
Effects of tobacco and neem leaves on blood parameters of cattle.

Group	TEC (million/Cu.mm)		Hb (%)		PCV (%)		ESR (mm/1 <sup>st</sup> h)	
	Day0	Day28	Day0	Day28	Day0	Day30	Day0	Day30
A	6.7±0.1 <sup>a</sup>	11.3±0.2 <sup>a</sup>	6.4±0.1 <sup>a</sup>	8.7 ± 0.2 <sup>a</sup>	29.5±0.2 <sup>a</sup>	30.4±0.5 <sup>a</sup>	0.1±0.0 <sup>a</sup>	0.03±0.1 <sup>a</sup>
B	6.5±0.1 <sup>a</sup>	10.6 ±0.2 <sup>a</sup>	7.1±0.1 <sup>a</sup>	8.6 ± 0.1 <sup>a</sup>	28.5±0.3 <sup>a</sup>	29.3 ±0.4 <sup>a</sup>	0.1±0.0 <sup>a</sup>	0.03±0.1 <sup>a</sup>
C	7.1±0.1 <sup>b</sup>	6.6±0.2 <sup>b</sup>	6.5±0.4 <sup>a</sup>	6.6 ±0.2 <sup>a</sup>	29.7±0.3 <sup>a</sup>	23.2±0.3 <sup>b</sup>	0.9±0.0 <sup>b</sup>	1.9±0.1 <sup>b</sup>

Data have been presented here as mean±SE, and values with different superscript in the same column are statistically significant (p<0.05).

## REFERENCES

- Akter M, Hassan MK, Ahammed M, Ali MA, Biswas GC, Islam MT and Ehsan MA (2016). Descriptive epidemiology of Stephanofilariasis (Hump sore) in cattle. *Bangladesh Journal of Veterinary Medicine*, 14(2):167-170.
- Al-Quraishy S, Abdel-Ghaffar F, Al-Rasheid KA, Mehlhorn J and Mehlhorn H. (2012). Effects of a neem seed extract (MiteStop®) on mallophages (featherlings) of chicken: in vivo and in vitro studies. *Parasitology Research*, 110:617-622.
- Baki MA and Dewan ML (1975). Evaluation of treatment of Stephanofilariasis (Humpsore) with Nегuvon (Bayer) by clinic-pathological studies. *Bangladesh Veterinary Journal*, 9:1-6.
- Brammer R and Gibson M (1996). Efficacy of Ivermectin and Neem leaves against Stephanofilariasis. *Journal of Veterinary Parasitology*, 7:67-69.
- de Novaes AP and Miyashita AT (2007). Stephanofilariasis in humans: occurrence and transmission mechanisms. *The Journal of the Brazilian Society of Tropical Medicine*, 40:250-252.
- Johnson SJ and Toleman MA (1988). Prevalence of stephanofilariasis in young *Bos indicus* cattle in northern Australia. *Veterinary Parasitology*, 29:333-339.
- Johnson SJ, Arthur RJ and Shepherd RK (1986). The distribution and prevalence of stephanofilariasis in cattle in Queensland. *Australian Veterinary Journal*, 63: 121-124.
- Mehlhorn H, Abdel-Ghaffar F, Al-Rasheid KA, Schmidt J and Semmler M (2011). Ovicidal effects of a neem seed extract preparation on eggs of body and head lice. *Parasitology Research*, 109:1299-1302.
- Miyakawa VI, Reis AC and Lisbôa JA (2008) Clinical features and diagnosis of Stephanofilariasis in dairy cows. *The Brazilian Journal of Veterinary Parasitology*, 1:172-174.
- Nooruddin M and Dey AS (1990). Further study on the prevalence and degree of severity of skin disease in domestic ruminants in Bangladesh. *Bangladesh Veterinarian*, 7: 75-81.
- Peter JH, Paul JB, Jeffrey MB, Charles HK, Edward JP and Julie J (2008). Helminth infections: The great neglected tropical diseases. *Journal of Clinical Investigation*, 118:1311-1321
- Phukut SC, Das M and Barkakoty MR (2005). Humpsore in cattle in Assam, *Journal of Veterinary Parasitology*, 19:19-22.
- Rahman MM, Mostofa M, Jahan MS and Kamal MAHM (2009) Comparative efficacy of Neem leaves and Ivermectin (Ivomec) against ectoparasites in calves. *Journal of Bangladesh Agricultural University*, 7:73-78.
- Rai RB, Ahlawat SP, Singh S and Nagarajan (2010). Levamisole hydrochloride: an effective treatment for stephanofilarial dermatitis (humpsore) in cattle. *Tropical Animal Health and Production*, 26:175-176.
- Rao DV, Singh I, Chopra P, Chhabra PC and Ramanujalu G (1986). In vitro antibacterial activity of Neem oil. *Indian Journal of Medical Research*, 84:314-316.
- Singh T and Patel BA (2015). Management of root-Knot nematode (*Meloidogyne incognita*) in bottle ground using different botanicals in pots. *Journal of Parasitic Disease*, 39:441-445.
- Soulsby E (1982). *Helminths, Arthropods and Protozoa of domesticated animals*. Eastbourne, United Kingdom: Baillière, Tindall & Cassell.
- Sujon MA, Mostofa M, Jahan MS, Das AR and Rob (2008). Studies on Medicinal Plants against Gastro intestinal Nematodes of Goats. *Bangladesh Journal of Veterinary Medicine*, 6:179-183.
- Watrelot-Virieux D and Pin D (2006). Chronic eosinophilic dermatitis in the scrotal area associated with stephanofilariasis infestation of charolais bull in France. *Journal of Veterinary Medicine*, 53:150-152.