



Effect of neem seeds (*Azadirachta indica*) against tick infestation in calves

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ABSTRACT

The effects of neem seeds against tick infestation in calves were investigated in this study. The clinical and hematological parameters of treated and non treated tick infested calves were also studied. Ivermectin was used as a reference acaricidal drug. The neem seeds were collected from local market of Mymensingh district and proceed for making dust and prepared aqueous solution to use as spray. A total of 15 calves were examined for the presence of ticks by physical examination and were divided into three equal groups as, Group A (infected control group), Group B (treated with neem) and Group C (treated with ivermectin). The number of tick, feeding efficiency skin lesion, body weight and blood parameters like hemoglobin (Hb), packed cell volume (PCV), total leukocyte count (TEC) and erythrocyte sedimentation rate (ESR) were investigated at pre treatment (0 day) and post treatment periods (7th, 14th, 21st and 28th day). Neem seed spray at 1% was found effective against tick infestation with 70% reduction of tick at 7th day post treatment and 100% reduction after 14th day post treatment. Ivermectin was found 100% effective as early as 7th day post treatment. The feeding efficiency was increased following treatment with both neem and ivermectin. The skin lesion due to tick infestation at pre treatment period was also cured and became shiny with increasing the post treatment period. The body weight of calves were significantly increased ($P < 0.05$) at 11.41% and 12.68% while treated with neem seed and ivermectin, respectively. The Hb content, PCV, TEC and ESR values were also significantly changed ($P < 0.05$) following treatment with both neem and ivermectin as compared to non treated control. The study suggests that neem as an alternative of chemical acaricide can be used against tick infestation in calves with health benefit of calves.

Key words: Neem seed, ivermectin, tick and calves

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INTRODUCTION

Bangladesh is an agriculture based developing country. Livestock is considered to be the backbone of Agriculture (Anon, 1985). The cattle population of Bangladesh is the major source of animal protein, draft power, milk, meat and hides. Cattle contributes 95% draft power for cultivation, 50% for rural transportation, 20% for fuel requirements, 20% for employment and 10% for manuring of arable lands (DLS, 1995).

Parasitic diseases are very common in Bangladesh (Hannan et al., 2001). Ectoparasites like mites,

ticks, lice, blowflies etc. play an important role as vector of deadly pathogens to animals producing various diseases. Among the ectoparasites tick is the most common ectoparasites of animal in Bangladesh. Ticks transmit diseases like Texas fever, anaplasmosis, theiliasis, and viral encephalomyelitis of animals (Islam et al., 1999). The damage is done by the tick causes irritation, inflammation, exudation of lymph which coagulates to form crusts. A considerable amount of blood loss in animal results in disturbances in feeding and improper digestion which may lead to retarded growth, loss of weight, decrease milk and meat production and damage to skin and wool.

Control measures of tick, in tropical and subtropical countries, is usually practiced by regular acaricide applications where the use of synthetic chemical acaricides is the main method of control. Ivermectin was safest and more effective chemical acaricide for the treatment of both endo and ectoparasites. However, as these acaricides are very expensive and are not available everywhere in rural areas, the farmers cannot afford to use to control tick infestations. Furthermore, some serious disadvantages of using these acaricides, notably the development of resistance in ticks against various acaricidal compounds and their harmful effects have been reported (Muro et al., 2003). Therefore, there is need for alternative tick control measures that are effective, safe and economically and environmentally acceptable.

Recently much interest in the field of medicinal plants has been grown throughout the world. Various kinds of herbal extract showed the larvicidal and acaricidal effects as reported by Chungsamarnyart et al., (1991). Several indigenous medicinal plants (Ata, Neem, Pineapple, Tobacco) have anthelmintics action and used against both ecto and endoparasites in Bangladesh (Mostofa 1983; Safique 1983; Mannan 1997; Nath 1983). Neem (*Azadirachta indica*) has proven to be effective against many insect pests and disease vectors of agricultural products (Wilps, 1986; Zebitz, 1986; Schmutterer, 1990). Neem has insect repellent properties and inhibits the development and growth in insects (Butterworth & Morgan, 1971), disrupt the mating and oviposition of insects and inhibits the hatchability of their eggs and moulting of their nymphs (Rembold et al., 1986; Sazena, 1993). The present study were undertaken to evaluate the effect of neem on clinical and hematological parameters during tick infestation in calves.

MATERIALS AND METHODS

The experiment was carried out for a period of 6 months from June to November, 2010 in the Department of Pharmacology in collaboration with Department of Physiology of Bangladesh Agricultural University Mymensingh.

Drugs

Pour on Ivomec® (Ivermectin pour on formulation, MSD) was purchased from local market, Mymensingh.

Preparation of Neem seeds spray

Neem seeds collected from botanical garden, BAU Campus and were taxonomically identified. The seeds were dried in the sun, crushed to a fine powder using a wooden mortar and pestle, sieved through a 30 mm mesh and dissolved in water (1 gm/100ml) and stirred for 2hr. It was kept overnight at 40C and the supernatant was collected.

Administration of drug

A total of 15 calves (between 3 to 6 months) infected with ticks were selected. The calves were divided into 3 equal groups (Group A, B & C) and each group consisting of 5 calves. Calves of group A was kept as infected control. The group C was treated with ivermectin pour on (200 mg/kg body weight) as reference drug. Group B was treated with neem seeds (spray) at 1%. All animals were treated at weekly intervals. After administration of ivermectin and spraying with neem, all the calves were closely observed for 28 days. The treated and control animals grazed together in an area which was heavily infested with adult and immature ticks. All calves were kept in animal shade, wheat bran, rice polish, maize and salt are mixed together and supplied to the calve up to 1-2 kg approximately daily. All the calves were allowed for free pasture grazing for 2-3 hours daily. Plenty of water was also provided to all calves.

Clinical investigations

The severity of infestation of tick was estimated by counting the number of tick in a selected area (5 cm × 4 cm (20 square cm in abdominal region) of the individual calf. The selected 20 square cm were marked with a permanent colour and the ticks within this area were counted at pre-treatment (0 day) and post-treatment (7th, 14th, 21st, 28th) period. The ticks were detected by visual examination of the calf. Ticks were

preserved in 70% ethanol prior to species identification using stereo microscopy

To observe the effects of the ivermectin and neem spray on body weight of each calf the body weight of each calf was recorded before and after treatment and the mean differences were compared with the weight gained by the calf in control group.

The changes in feeding efficiency and hair coat of treated and untreated groups were compared at pre-treatment and post-treatment periods.

Hematological investigations

Blood samples were collected from jugular vein of the calves of treated and control group in vials containing anticoagulant (Sodium Citrate 3.8%) at day 0, 7th, 14th and 28th of treatment period and proceed for analyzing hematological parameters such as Total Erythrocyte Count (TEC), Hemoglobin (Hb) Content, Packed Cell Volume (PCV) and Erythrocyte Sedimentation Rate (ESR).

The hemoglobin estimation was performed by the acid Haematin method with the Hellige Hemometer (Coffin, 1955). The counting and calculation of red blood cells were performed as per methods indicated by Coffin (1955). The PCV was determined as per method described by (Coffin, 1955). The ESR value was determined as per methods describe by (Coffin, 1955).

Statistical analysis

Data obtained from the experiment on body weight, hematological parameters such as Hb, PCV, TEC, ESR were analyzed statistically between control and treated groups by using STUDENT 'T' test.

RESULTS AND DISCUSSION

Hematological estimation in blood serum of treated and non treated calves was conducted to know the systemic effect of these drugs on tick infested animal.

Table 1

Effect of treatment on tick infestation, feeding efficiency and hair coat lesion

	Tick (number) in day of treatment					Feeding efficiency in day of treatment					Hair coat lesion score in day of treatment				
	0	7	14	21	28	0	7	14	21	28	0	7	14	21	28
Control	20	20	23	25	30	+++	++	++	+	+	1	1	2	2	3
Invermectin	20	0	0	0	0	+++	+++	+++	++++	++++	1	1	0	0	0
Neem seeds	20	14	0	0	0	+++	++	+++	++++	++++	1	1	1	0	0

+ (severely decrease); ++ (decrease) +++ (normal); ++++ (increase). 0=no lesion; 1=mild lesion; 2=moderate lesion; 3=severe lesion

Neem seeds at 1% spray were found to be 70% effective against tick infestation on post-treatment day 7 and the number ticks were 0 within the selected area on 14th, 21st and 28th day after the treatment whereas in control group the number of ticks increased gradually on those days (table 1). Neem seeds spray in this study was found more potent than the study by Rahman et al., 2009 where they used neem leaf at 15% spray. Ivermectin is 90%- 100% effective against tick infestation in calves reported by Ninkov and Savin

(1986), Pedroso et al. (1994); Thompson et al. (1994), Titcherer et al. (1994) and Sangwan et al. (1995).

Ivermectin was used in this experiment as reference acaricidal drug. Ivermectin was found 100% effective against tick infestation after 7 days post treatment. The feeding efficiency was increased in the treated groups whereas the efficiency was gradually decreased in non treated control group might be due to feeding of blood and

annoyance by increased tick infestation. The rough body coat before treatment was become smooth

and shiny following treatment with neem seeds spray and ivermectin pour on (table 1).

Table 2
Effects of body weight (kg) in calves

Treatment	Pre-treatment		Post-treatment		
	'0' day	7 th day	14 th day	21 st day	28 th day
Control	17.24±0.167	17.20±0.16	17.52±0.19	17.64± 0.10	18.78±0.17 (8.22)b
Neem seeds	18.41±0.087	18.41 ± 0.14	19.63±0.11*	19.52±0.20*	20.80±0.80* (11.41)a
Ivermectin	18.52±0.28	18.52± 0.11	19.184±0.11*	19.59±0.16*	21.21±0.08 (12.68)a

Values given above are mean±SE of 5 calves, * significantly increased (P<0.05), a = Percent increased, b = Percent decreased

The body weight of parasitic infested calves was increases after administration of neem seeds spray. The body weight was decreased in non treated infected control group due to decrease feeding efficiency, blood loss and stress, reaching 8.22% weight loss at the 28th day of post treatment. The body weight was significantly increased (P<0.05), reaching 20.80±0.80 % gm at 28th days post-

treatment It might be due to proper digestion, absorption and metabolism of feed-nutrient because of absence of tick infestation. Similar results were found by Rahman et al., 2009 where they used neem leaves. Following ivermectin treatment the body weight was significantly increased at 28th days post treatment, reaching slightly higher than that of neem seed.

Table 3
Effects on Hemoglobin content (Hb) (gm %) in calves

Treatment	Pre-treatment		Post-treatment		
	'0' day	7 th day	14 th day	21 st day	28 th day
Control	6.6±0.42	7.08±0.20	6.63±0.18	6.89±0.19	6.74±0.17 (2.03)
Neem seeds	6.8±0.12	7.38±0.12*	7.66±0.11*	8.60±0.15*	8.7±0.11* (21.83)a
Ivermectin	6.46±0.14	7.32±0.10*	7.56±0.15*	8.34±0.13*	9.0±0.20* (28.22)a

Values given above are mean±SE of 5 calves, * significantly increased (P<0.05), a = Percent increased, b = Percent decreased

Hemoglobin content was increased significantly (P<0.05) on 21st and 28th day of treatment, reaching 8.604±0.15 gm % and 8.7±0.11 gm % respectively. PVC was significantly increased on 28th day of treatment. The mean value of haemoglobin was decreased in the tick affected control group. The results are in agreement with the reports of Nettleton and Beckett (1976) and Anosa (1977). TEC and ESR were significantly increased and decreased respectively after 14th days of treatment and continue up to end of the

experiment (table 3). Although the Hb content did not significantly changes in non treated control group but it was significantly increased to the extent of 21.83% and 28.22% in neem seed and ivermectin treated groups. The Hb content was found higher than the treatment with neem seed after 21st days post treatment. PCV (%) was found comparatively lower in ivermectin treated calves than calves treated with neem seed spray. TEC count was slightly higher in ivermectin treated calves and ESR values were similarly decreased as

neem seed spray following treatment with ivermectin. Similar results were observed when neem leaf was used in a study of Rahman et al., 2009.

Table 4
Effects on PCV (%) in calves

Treatment	Pre-treatment	Post-treatment			
	'0' day	7 th day	14 th day	21 st day	28 th day
Control	28.99±0.91	29.70±0.26	29.2±0.25	26.44±0.27b	23.20±0.30 (19.97)b
Neem seeds	30±0.31	29.58±0.23*	29.49±0.16*	30.70±0.43*	31.36±0.47* (4.26)a
Ivermectin	27.41±0.35	28.51±0.32*	28.89±0.17*	29.82±0.89	29.50±0.381 (7.11)a

Values given above are mean±SE of 5 calves, * significantly increased (P<0.05), a = Percent increased, b = Percent decreased

The percent PCV was significantly increased following treatment with neem seed whereas the percent PCV was decreased after 21 day of post treatment in non treated control group. Ivermectin treated group showed a slightly increased the

percent PCV. In the present study significant changes in Hb gm% and PCV% were observed in the treated group of calves and this might be due to expulsion of blood sucking by tick from the body.

Table 5
Effects on TEC (million/cu. mm) in calves

Treatment	Pre-treatment	Post-treatment			
	0 day	7th day	14th day	21st day	28th day
Control	7.36±0.11	6.72±0.17	6.46±0.15	6.38±0.13	6.70±0.15 (8.96)b
Neem seeds	6.59±0.16	7.48±0.07	8.91±0.21*	10.07±0.08	10.00±0.19* (26.40)a
Ivermectin	6.0±0.09	8.32±0.13*	9.05±0.07*	10.36±0.16*	11.00±0.17* (45.45)a

Values given above are mean±SE of 5 calves, * significantly increased (P<0.05), a = Percent increased, b = Percent decreased

Table 6
Effects on Erythrocyte sedimentation rate (ESR mm/1st hr)

Treatment	Pre-treatment	Post-treatment			
	0 day	7th day	14th day	21st day	28th day
Control	0.97±0.019	1.03±0.012 (9.28)	1.40±0.02 (26.35)	1.83±0.03 (23.19)	1.20±0.04 (14.16)a
Neem seeds	0.128±0.011	0.09±0.01(41.69)	0.06±0.01*(42.33)	0.05±0.01 (20.00)	0.05±0.01*(64.06)b
Ivermectin	0.150±0.010	0.11±0.01(30.91)	0.09±0.02*(20.09)	0.08±0.01*(10.36)	0.04±0.01*(76.67)b

Values given above are mean±SE of 5 calves, * significantly increased (P<0.05), (%a) = Percent of increased a, (%b) = Percent of decreased b.

The TEC was decreased gradually with increasing the day of infestation, reaching 8.96%

decreased TEC at 28th day in control group. Neem seeds and ivermectin showed gradual

increasing of TEC with a significant increased value of 26.40 and 45.45 million/cu. mm TEC, respectively at 28th day post treatment.

The significant reduction in the sedimentation rate of erythrocytes in treated group was observed and this may be due to recovery from inflammation produced by tick.

The present study is comparable with the study of Rahman et al., 2009 where they used ivermectin as injectable formulation and neem leaves instead of ivermectin pour on and neem seed. However, present research suggests that neem seeds spray is potent in its effect against tick infestation with health benefit in calves compared to neem leaf spray. Further research is necessary to determine the drawl periods, pharmacokinetic and toxicological parameter in this regard.

REFERENCES

- Anon (1985). Asian Livestock, Vol. X, No. 5, P. 49.
- Anosa VO (1977). Hematological observations on helminthiasis caused by haemonchus contortus in Nigerian dwarf calves. Bulletin of Tropical Animal Health and Production in Africa, 9: 11-17.
- Butterworth JH and Morgan ED (1971). Investigation of the locust feeding inhibition of the seeds of the neem tree, *Azadirachta indica*. Journal of Insect Physiology. 17: 969-977.
- Chungsamarnyart N and Jansawan W (2001). Effect of Tamarindus indicus L. against the Boophilus microplus. Kasetsart Journal of Natural Science. 35(1): 34-39.
- Coffin DL (1955). Manual of Veterinary Clinical Pathology. Third Ed. Coinstock Publishing Associates. Inc. Ithaca New York, 116-157.
- DLS (1995). Personal communication Directorate of Livestock Services, Bangladesh.
- Hannan ASMA, Mostofa M, Hoque MA, Alim MA (2001). Efficacy of ivomec pour-on against gastrointestinal nematodes, lice and ticks in goats, Bangladesh Veterinarian, 18 (2): 95-98.
- Islam, MK and Kibria AKMG, Majumder S, Islam, MF and Mondal MMH (1999). Factors influencing Ixodid tick (Acari: Ixodidae) infestation of cattle in Bangladesh. Progressive Agriculture, 10: 131-135.
- Mannan MA, Rafiq K, Mostofa M and Hasan Q (1997). Comparative efficacy of Ivermectin, Neguvon and Ata-tobacco Herbal Preparations against naturally occurring humpsore lesions in cattle. Bangladesh Veterinary Journal 31(3-4): 119-122.
- Mostofa M (1983). Efficacy of some indigenous medicinal plants against gastro-intestinal nematodiasis in cattle and their comparative activity with that of Nemafox. M. SC (vet. Sc) Thesis, Dept of Pharmacology, BAU. Mymensingh.
- Muro CEF, Cruz VC, Fernandez RM, Molina TJ, Soria CG and Ramos PM (2003). Repellence of Boophilus microplus larvae in *Stylosanthes humilis* and *Stylosanthes lamata* plants. Parasitologia Latinoamericana. 58(3/4): 118-121.
- Nath K, Rajagopal and Garg AK (1983). Water washed Ata seed kernel cake as a cattle feed. Journal of Agricultural Science 101(2): 323-326.
- Nettleton P and Beckett P (1976). Haematology of indigenous goat in Swaziland. Bulletin of Tropical Animal Health and Production in Africa, 8: 60-61.
- Pedroso PD, Moya BGE, Borja GEM, Paiva D and Pedroso DE (1994). Ivermectin for the control of Sarcopromusca pruna (Shannon and Del Ponte, 1926). (Diptera; Muscidae), carrier of Dermatobia Homini, L.Jr. (1781). (Deptera; Cuterebridae). Revista Barsileira de Parasitologia Veterinaria. Publication 1995, 3(1): 61-64.
- Rembold H, Forster H and Czoppoelt CH, 1986. Structure and biological activity of Azadirachtins A and B. Proc. 3rd Int. Neem Conf. Nairobi, Kenya. pp. 149-160.
- Sazena RC (1993). Scope of neem for developing countries. Proc. Wld Neem Conf., Febr. 1993, Bangalore, India. pp. 30-36.
- Safique MI (1983). Studies of anthelmintic effects of pineapple (Ananus sativus) leaves on calves. M. Sc. (Vet. Sc.) thesis, Submitted to

- Department of Medicine, BAU, Mymensingh.
- Sangwan RP, Langholef WK, Cramer LG and Eagleson JS (1995). An ivermectin tablet for calves; efficacy against gastrointestinal nematodes and a bioavailability comparison with a liquid ivermectin formulation. *Veterinary Parasitology*. 60(3/4): 297-302.
- Schmutterer H, (1990). Properties and potential of natural pesticides from the neem tree. *Annual Report of Entomology*. 35: 77-297.
- Thompson DR, Kugg D, Scott PG, Cramer LG and Barrick RA (1994). Rainfall and breed effects on the efficacy of Ivermectin jetting fluid for the prevention of fly strike and treatment of infestation of lice in long woolled calves. *Australian Veterinary Journal* 71(6): 161-164.
- Titcherer RN, Parry JM and Grimshaw WTR (1994). Efficacy of formulation of abamectin, ivermectin and moxidectin against sucking and biting lice of cattle. *Veterinary Record*. 134(17): 452-453.
- Wilps D (1986). Growth and adult moulting of larvae and pupae of the blowfly, *Formia terae-novae* in relationship to Azadirachtin concentration. *Proc. 3rd Int. Neem Conf. Nairobi*, pp. 299-324.
- Zebitz CPW (1986). Potential of neem seed kernel extract in mosquito control. *Proc. 3rd Int. Neem Conference*. pp. 555-573