

Prevalence and Therapeutic Efficacy of Anthelmintic against *Neoscaris vitulorum* in Buffalo Populations from Sylhet District of Bangladesh

Shishir Kanu¹, Md. Shahidur Rahman Chowdhury¹, Md. Abdus Sabur¹, Md. Mahfujur Rahman¹, Kazi Mehetazul Islam², Md Bashir Uddin¹, Md. Rafqul Islam¹ and Md. Mukter Hossain^{1,*}

¹Department of Medicine, Faculty of Veterinary, Animal and Biomedical Sciences, Sylhet Agricultural University, Sylhet-3100, Bangladesh

²Department of Parasitology, Faculty of Veterinary, Animal and Biomedical Sciences, Sylhet Agricultural University, Sylhet-3100, Bangladesh

Abstract: Background and Objectives: The epidemiological features of *Neoscaris vitulorum* infection in buffaloes are important to know their transmission and risk factors assessment as well treatment regimens. This study was conducted with the objectives of determining the prevalence of *Neoscaris vitulorum* infection in buffaloes in Sylhet district of Bangladesh and to evaluate the efficacy of anthelmintics against the parasite.

Materials and Method: A total of 211 fecal samples were collected from buffaloes and examined for the detection of parasitic eggs during the period from July 2017 to June 2018. For the determination of therapeutic efficacy, animals were randomly divided into 6 groups (T₀, T_i, T₁, T₂, T₃, T₄) where each group consisting of 3 buffaloes. The group T₀ (negative control-non infected) and T_i (positive control-infected) were treated only with phosphate buffer saline (PBS). The other four infected groups were treated as T₁ with albendazole, T₂ with piperazine citrate, T₃ with ivermectin and T₄ with combination of tetramisole HCL and oxclozanide. Before trials day 0 and after treatment of 7th, 14th, 21st and 28th days fecal samples examined using McMaster technique.

Results: The overall prevalence was estimated 17.06% (36/211). The prevalence was higher among buffalo calves of below six months (39.28%) followed by 6 months to 1 year, 1 to 2 years and above 2 years as 25%, 22.5% and 7.20% respectively. The infection rate was high in females (20.0%) than males (13.18%). The parasite was more prevalent in rainy (21.42%) than summer (16.67%) and winter (14.29%) seasons. The EPG results revealed piperazine citrate was the most effective (100%) against *Neoscaris vitulorum*. The percent efficacies of other anthelmintic used in this study were albendazole, ivermectin and combination of tetramisole HCL and oxclozanide as 95.49%, 86.20% and 95.58% respectively.

Conclusion: The prevalence of *Neoscaris vitulorum* infection in buffalo at Sylhet district of Bangladesh was relatively high. The results revealed that piperazine citrate, tetramisole HCL and oxclozanide combination are highly effective for the reduction of egg per gram (EPG) of *Neoscaris vitulorum* infection in buffalo. On the other hand ivermectin and albendazole were also around ninety percent effective respectively.

Keywords: *Neoscaris vitulorum*, prevalence, therapeutic efficacy, buffalo.

INTRODUCTION

Buffalo is one of the most important species of livestock as a source of dairy, meat, manure and drought in Bangladesh. Both swamp and river type buffaloes are found in Bangladesh [1]. The amount of meat, milk and income reduced because of factors such as death and sickness [2]. *Neoscaris vitulorum*, a nematode (round worm) of *Bubalus* and *Boss* species, founds mostly in tropical and subtropical climates throughout the world [3]. It is considered to be a major limiting factor in farms, due to high morbidity, mortality, loss of production and ineffective implementation of breeding programs [2]. It is among

the most destructive parasites of calves, the larvae of which undergo migration causing great damage to many organs, especially the liver and the intestine. It is responsible for up to fifty percent mortality in cattle and buffalo calves [4]. This parasite is mainly transmitted through trans-placental and trans-mammary transmission, causing the disease characterized by severe diarrhea, weight loss and anorexia, particularly in buffalo calves between 1 and 3 months [5]. The disease in calves is the top most cause of morbidity and mortality [6]. Neoscariasis can be diagnosed on the basis of clinical signs, necropsy findings, fecal examination for eggs and serological tests. When the cow is pregnant the larvae migrate from the liver to the mammary gland and prior to parturition, to the milk through which the calf is infected [7]. An adult female parasite produces thousands of eggs daily. Egg fecundity ranges from 8000 to 100,000 eggs per gram

*Address correspondence to this author at the Department of Medicine, Faculty of Veterinary, Animal and Biomedical Sciences, Sylhet Agricultural University, Sylhet-3100, Bangladesh; Tel: +88-01712054850; E-mail: mukter.vetmed@sau.ac.bd

(EPG) feces per day [8]. When a host ingests infective stage eggs, the larvae hatch out in the intestinal tract and migrate to the liver, lungs and other tissues [9]. A significant number of infections are fatal and ingestion of large number of eggs resulted in paralysis, conjunctivitis and opisthotonos. Higher prevalence of *Neoscaris vitulorum* was found up to 6 month of age [10]. Treatment of buffalo calves at different times after birth demonstrated that transmission from cow to the calf via milk occurs in all calves during the first 2 days after birth, decreases to 53% by 6 days, 10% by 8-9 days and 2% from day 10 onwards [11]. Without treatment, the prevalence may reach 100% in calves, with mortality rates up to 80% as a result of weakening and dehydration [12]. Numerous broad spectrum anthelmintic are effective against adult worms and larvae in the gut (albendazole, febendazole, oxfendazole etc.). A few other narrow spectrum anthelmintic such as morantel, pyrantel and piperazine derivatives are effective against adult worms but may not control larvae and other roundworm species that often infect livestock simultaneously with *Neoscaris vitulorum* worms. In developed countries, the data on epidemiology of *Neoscaris vitulorum* are published in an efficient manner as an aid to combat infections more effectively. In contrast, in developing countries, little published information exists and data on the epidemiological aspect of *Neoscaris vitulorum* infections. Few researches related to gastrointestinal parasites in buffaloes have done in some regions of Bangladesh [13]. Very few numbers of epidemiological works on *Neoscaris vitulorum* in buffaloes has been done. This study was undertaken to determine the prevalence of *Neoscaris vitulorum* in buffaloes based on different age, sex, and seasons as well as to evaluate the therapeutic efficacy of different of anthelmintic against *Neoscaris vitulorum* infection.

MATERIALS AND METHODS

Study Area and Period of Study

The study was conducted in Sylhet district of Bangladesh. It was located in North-East part of Bangladesh 24°30" North latitude and 91°40" East longitude. The average maximum and minimum temperatures were 34°C and 13°C, respectively. The annual average rainfall was 3854.2 mm and humidity was 68.92%. A lot of rivers are passing away through Sylhet district making natural pasture lands, which are suitable for buffalo rearing. The research work was conducted for a period of one year from July 2017 to June 2018.

Collection of Fecal Samples

During the study period, a total of 211 fecal samples from same number of household buffalo were collected from different places in the district. The fecal samples were collected directly from the rectum of buffaloes. Suitable containers like screw-capped wide mouthed glass bottles were used for sample collection and then transported to the laboratory for further examination. The sample collected from the remote areas was preserved in 4% formalin and then transport to the laboratory. Twenty (20) g of feces were collected from each buffalo and kept at 4°C before examination. Identification was performed based on morphology of *Neoscaris vitulorum* eggs as described [14].

Examination of Fecal Samples

The color, consistency, presence of blood, mucus, dead worms and others was looked before microscopic examination by naked eye. Microscopic examination of feces for detecting *Neoscaris vitulorum* eggs may vary from a simple direct smear to more complex methods involving centrifugation and McMaster techniques.

Examination by Direct Smear Method

This method was found useful only in the cases of heavy infections, small portion of fecal sample was directly placed on a grease free slide by a glass rod and 1-2 drop of saline was added to make a uniform suspension and then a cover slip was examined under low power microscope (10X).

Examination by Indirect Method

The purpose of methods is to detect light infections, to save time by concentrating the eggs in a small volume. In the Willis method about 1ml of mixed fecal specimen is diluted with 10-20 ml of saturated common salt solution in a suitable narrow cylinder, which is filled the top with the saturated salt solution. A clean slide cover glass was slide ways over the top of the cylinder so that it is in contact with the saturated salt solution. After about 10-20 minutes the slide or cover glass was quickly removed and examine under a low power of microscope [16].

Determination of the Efficacy of Different Anthelmintic against *Neoscaris vitulorum* Infection

Selection of Experiment Site

The highest number of buffalo population, high prevalence of neoscaris infection, good cooperation of

the Veterinary were the criteria behind the selection. More than three quarter of the district consists of mostly high plain, water logged and low lying areas. In addition, the semi-scavenging systems rearing of buffalo were the most popular with the aim of having subsistent family income. The semi-scavenging buffaloes were feed on the grasses.

Selection of the Experimental Buffaloes

Household farms were selected randomly from different places. All buffaloes belonging to the 6 household farms were brought under parasitic screening. Up to 20 g of feces per buffalo was taken aseptically from the rectum. The direct smear methods as previously described [16] followed by the modified McMaster counting technique described earlier were used to screen *Neoscaris vitulorum* eggs in this study.

Grouping of Experimental Buffaloes

The buffaloes were randomly divided into 6 groups, each consisted of 3 buffaloes. *Neoscaris* infected specific treatment groups were selected by tagging a coin. The group was designated as T₀, T_i, and T₁ to T₄.

Experimental Trials

The group T₀ (negative control-non infected) and T_i (positive control-infected) were treated only with PBS. T₁ to T₄ were treated with different anthelmintic. Schedules are presented in Table 1.

Fecal Samples Collection and Egg Counting after Treatment

Before trials (day 0) fecal samples were collected for parasitic egg count from the experimental buffaloes. After that weekly EPG count was done on day 7th,

14th, 21st and 28th post treatment. Fecal egg counts were done by McMaster technique. This method was based on the principle that the eggs floated up in a counting chamber. A special type of slide devised by McMaster is required in this technique.

McMaster Egg Counting

This was done by modified McMaster counting chamber as described [15]. This technique is useful in the determination of number of eggs per gram (EPG) of feces. Briefly 3g of fecal samples were taken and 42 ml of flotation solution was added to the sample and then thoroughly mixed so that it forms a homogenous mixture. The solution was then transferred through a sieve in order to remove the coarse particles and filled in test tubes and centrifuged at 2000 rpm for 2 minutes. The supernatant was poured off and the sediment was agitated and again the tube was filled to the previous level with flotation solution. The upper fluid portion was poured in both chambers of McMaster slide with the help of pipette and no fluid was left in the pipette, as the eggs would rise quickly in the floatation fluid. Both chamber of fluid examined and number of eggs was multiplied by 100 to determine EPG.

Statistical Analysis

The analysis was performed by using statistical software SPSS (Version 17.0, SPSS Inc., Chicago, IL, USA) and Microsoft Excel 2007. Values of P<0.001 and P<0.05 were considered as significant at 99.99% and 95% confidence, respectively.

RESULTS

A total of 211 fecal samples were collected from buffaloes and examined for detection of *Neoscaris*

Table 1: Infection Status and Treatment Schedule of Naturally Infected Experimental Buffaloes

Group of buffaloes	Status of infection	Treatment regimens	Trade name of anthelmintics	Manufacturer company	Doses	Routes of administration
T ₀	Non-infected	PBS	-	-	-	Orally
T _i	Infected	PBS	-	-	-	Orally
T ₁	Infected	Albendazole	Alben®-vet (bolus)	Eskayef Pharmaceuticals Ltd., Bangladesh	7.5 mg/kg bwt ^a	Orally
T ₂	Infected	Piperazine- citrate	Peravet® (powder)	The ACME Laboratories Ltd., Bangladesh	200 mg/kg bwt ^a	Orally
T ₃	Infected	Ivermectin	I-ver® (injection)	The Globe Pharmaceuticals Ltd., Bangladesh	0.5 ml/25 kg bwt ^a	^b S/C injection
T ₄	Infected	Tetramisole and Oxytoclosanide	Levanid® (bolus)	The ACME Laboratories Ltd., Bangladesh	7.5 mg/kg bwt ^a	Orally

^abwt = body weight; ^bS/C = subcutaneous.

Table 2: Prevalence of *Neoscaris vitulorum* Infection in Buffaloes

Variables	No. of animal examined	No. of positive animal	Prevalence
Age			
Up to 6 months	28	11	39.28%
6 months-1 year	40	10	25.0%
1-2 years	31	7	22.5%
Above 2 years	111	8	7.20%
Total	211	36	17.06%
Sex			
Female	120	24	20.0%
Male	91	12	13.18%
Total	211	36	17.06%

Table 3: Seasonal Prevalence of *Neoscaris vitulorum* Infection in Buffaloes

Name of seasons	Total no. of animals examined	No. of affected animals	Prevalence
Summer	78	13	16.67%
Rainy	56	12	21.42%
Winter	77	11	14.29%
Overall	211	36	17.06%

Table 4: Egg Per Gram of Feces in Experimental and Control Group of Buffaloes after Treatment

Experimental group	Day 0	Day 7	Day 14	Day 21	Day 28
T ₀	0	0	0	0	0
T _i	746±20.39	772±20.83*	800±18.17*	812±18.28*	826±18.6*
T ₁	733±88.22	533±57.33*	366±66.66*	200±57.73*	33±37.12*
T ₂	966±88.18	533±66.67*	233±33.33*	66±33.33*	0*
T ₃	464±23.69	178±18.4*	135±14.28*	121±18.45*	64±9.22*
T ₄	770±51.47	367±23.85*	185±6.52*	121±6.78*	34±2.21*

*Significant at 1% level (P < 0.01).

vitulorum eggs. The overall prevalence of the infection was estimated 17.06% (36/211). The prevalence was higher among buffalo calves of below six months (39.28%) followed by 6 months to 1 year, 1 to 2 years and above 2 years as 25%, 22.5% and 7.20% respectively. The infection rate was high in females (20.0%) than males (13.18%). The parasite was more prevalent in rainy (21.42%) than summer (16.67%) and winter (14.29%) seasons. Before trials day 0 and after treatment of 7th, 14th, 21st and 28th days fecal samples examined using McMaster technique. The EPG results revealed piperazine citrate was the most

effective (100%) anthelmintic against *Neoscaris vitulorum*. The percent efficacies of other anthelmintic used in this study were albendazole, ivermectin and combination of tetramisole HCL and oxclozanide as 95.49%, 86.20% and 95.58% respectively.

The efficacies of the products were evaluated on the percentage of reduction in mean egg count after treatment compared to the mean EPG before treatment. A significant (P < 0.01) reduction of EPG count was found on 7th, 14th, 21st, 28th day of treated buffaloes of group T₁ to T₄ respectively.

Table 5: Determination of Therapeutic Efficacy of Different Anthelmintic against *Neoscaris vitulorum* in Buffaloes

Treatment group	Percentage of EPG reduction			
	Day 7	Day 14	Day 21	Day 28
T ₁ (Albendazole)	27.28%	50.06%	72.71%	95.49%
T ₂ (Piperazinecitrate)	44.82%	75.87%	93.16%	100%
T ₃ (Ivermectin)	61.63%	70.70%	73.92%	86.20%
T ₄ (Tetramisole and Oxytocosanide)	52.34%	75.97%	84.29%	95.58%

DISCUSSION

A total of 211 numbers of fecal samples were examined for the presence of *Neoscaris vitulorum* infection. 36 samples were found positive for *Neoscaris vitulorum* infection, giving an overall prevalence of 17.06%. Higher incidence of *Neoscaris vitulorum* infection in buffalo calves have also been reported from different parts of India and abroad [16-21]. The variation in the prevalence rate of the *Neoscaris vitulorum* infection may be associated with different factors like different geo-climatic conditions, poor hygienic condition of the shed, health care management and sample variation.

Among age of animals, highest incidence was recorded in the age group of < 0.5 year followed by 6 months to 1 year, 1 to 2 and above 2 years. Similar findings were reported by others [22-23]. The cause for higher incidence of *Neoscaris vitulorum* infection in the younger age in the present study may be due to pre-natal infection i.e. trans-placental infection at the later stage of pregnancy and transfer of 3rd stage larvae in colostrum and milk or post-natal infection due to poor managemental condition. The lower rate of incidence in older calves may be due to elimination of worm burden or to immunity gained by the animals from repeated infection with the infective larvae through colostrum or milk [14].

Among samples, 120 were from female and 91 were from male buffaloes. Higher prevalence of *Neoscaris vitulorum* infection was recorded in female calves than male calves. Similarly higher prevalence in female calves than male buffalo were reported [17, 21]. The sex of the buffalo calves had no significant influence on the incidence rate [23] while others reported higher incidence in males than females [21, 24]. Though absolute reason for higher infection in

females has not been well explained by previous workers, it is commonly believed that, since female calves are allowed to suckle frequently and more milk than that of male calves, they are more exposed to higher risk of infection through trans-mammary route. The reason for slightly prevalence in females than males the present study may also be attributed to the above fact and variation in the number of fecal samples examined.

Prevalence of *Neoscaris vitulorum* infection was recorded be highest during rainy season followed by summer and winter. Similar findings were also recorded [16, 18, 25-28] in buffalo calves. Higher incidence in rainy season may be due to higher rainfall and relative humidity that favors the maximum embryonation, development and dissemination of the exogenous stage of the parasite. High rainfall provides suitable molarities of salt present in the soil, which is an important factor for ecdysis [14].

The group T₀ (negative control-non infected) and T_i (positive control-infected) were treated only with PBS. Other four groups (T₁, T₂, T₃, T₄) were treated with different types of anthelmintics. This study showed the therapeutic efficacy of different types of anthelmintics. The number of eggs gradually decreased T₀ (negative control-non infected) and T_i (positive control- infected) EPG values were increased. The result of the comparative efficacies of different anthelmintics showed that piperazine citrate, tetramisole HCL and oxytocosanide combination against *Neoscaris vitulorum* in naturally infected buffaloes were highly effective. These results are similar to [29] report 99.9% efficacy of piperazine against neoscaris infection in buffalo calves. piperazine citrate was 100% effective against ascarid worms in buffalo [30]. A single administration of Levamisole at the age of ten days killed the adult and immature intestinal worms and prevented

recontamination of the environment and the efficacy of pyrantel and febendazole was 100% and piperazine 57% against *Neoscaris vitulorum* infection in buffalo [11]. Another study of the therapeutic efficacy of ivermectin, and albendazole against *Neoscaris vitulorum* infection in buffalo was recorded to be 93.33% and 84.61% respectively [25] which is similar in case of albendazole but in case of Ivermectin the efficacy was slightly lower. The findings of the present study reveal that piperazine citrate, tetramisole HCL and oxclozanide combination are highly effective for the reduction of EPG of *Neoscaris vitulorum* infection in buffalo. On the other hand ivermectin and albendazole are also effective against *Neoscaris vitulorum* infection.

CONCLUSION

This study concluded that the overall prevalence of *Neoscaris vitulorum* infection in buffalo at Sylhet district of Bangladesh was relatively high. The findings of this study revealed that piperazine citrate, tetramisole HCL and oxclozanide combination are highly effective for the reduction of EPG of *Neoscaris vitulorum* infection in buffalo. On the other hand ivermectin and albendazole was also around ninety percent effective. The findings of the present study may help the future researchers to explore the details and wide therapeutic uses in Bangladesh for the treatment and control of *Neoscaris* infestation in buffaloes. Further studies on the prevalence of *Neoscaris vitulorum* infection would clarify the efficacy of the anthelmintics widely used in different animal species and livestock management system in Bangladesh.

ACKNOWLEDGEMENT

The authors gratefully express their gratitude to Sylhet Agricultural University Research System (SAURES) for financial support to complete this research successfully.

CONFLICT OF INTEREST

The authors declare that there is no conflicting interest with regards to the publication of this manuscript.

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Received on 08-02-2021

Accepted on 29-03-2021

Published on 13-04-2021

<https://doi.org/10.6000/1927-520X.2021.10.03>

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