Original Research Article

Comparative Study on Therapeutic Efficacy of Different Anthelmintics in Treatment of Swamp Buffalo Naturally Infested with Gastro-intestinal Parasites

ABSTRACT

The present study aimed to evaluate the comparative therapeutic efficacy of different modern anthelmintics like albendazole, levamisole, the combination of tetramizole and oxyclosanide; levamisole and triclabendazole against gastrointestinal (GI) parasites in buffalo. For this purpose, a total of 100 buffalo were selected which were suspected to suffer from single or mixed parasitic infestation. These 100 buffalo were divided into 5 equal groups (A, B, C, D, and E) each of which contained 20 buffalo, and buffalos of group E were kept as a control group. Four types of modern anthelmintics bolus from 4 different renowned companies in Bangladesh were used and administrated orally. All 5 groups of buffalo were observed for 28 days before treatment (0 days) and after treatment (7^{th}, 14^{th}, 21^{st}, and 28^{th} day) with 4 drugs. Fecal samples were collected from 100 buffalo and were examined for the determination of eggs per gram of feces (EPG) by the McMaster technique. Collected data were entered into Microsoft Excel Worksheet for performing descriptive statistics to calculate mean and standard error among the four anthelmintics preparations. P-value was determined by computer using a statistical package programmed SPSS. The temperature of the treated and control buffalo was almost within normal range before and after treatment. Among the four drugs, the highest efficacy was found in the case of Trilev vet® (levamisole+triclabendazole) which reduced EPG count to 87.61%. The lowest efficacy was found in the case of Endokil® (albendazole) which reduced the EPG count to 42.99% on the 28^{th} day. From the findings, it was clear that the mean of the four drugs was dissimilar in efficacy, and thereby the Trilev vet(R (levamisole+triclabendazole) performed better than the other three.

Keywords: Anthelmintics, swamp buffalo, parasites, infestation, EPG.

1. INTRODUCTION

Buffaloes are considered one of the most important sources of milk and meat production [1, 2]. However, they can become infested with different types of parasitic gastro-intestinal worms resulting in serious effects and health deterioration, leading to substantial economic losses [3, 4]. Livestock plays an important role in the agricultural economy of Bangladesh. According to the Department of Livestock Services (DLS) of Bangladesh, the contribution of the livestock sector to the gross domestic product (GDP) is 1.90% with GDP growth rate of livestock at 3.10% [5]. Indigenous livestock breeds are known for their hardiness, disease resistance, survival on little inputs, and adaptability to variable environments [6]. Of all

domestic animals, the Asian buffalo holds the greatest promise and potential for production [7]. Buffalo plays an important role in farmers' economic life in Bangladesh. About 80% people of Bangladesh live in the village and most of them are fully or partially dependent on agriculture. Buffalo has a significant contribution to GDP through the production of meat, milk, and skin representing about 27.0%, 23.0%, and 28.0%; respectively to the total production from the livestock sector in Bangladesh [8]. According to the estimate of the Bangladesh Bureau of Statistics (BBS) 2021-22, the current buffalo population in Bangladesh is about 1.5 million [9]. Of the total buffalo population of the country 42.8%, 39.9%, and 11.8% of buffaloes are found in the sugarcane belt, coastal areas, and marshy land, respectively [10]. In Sylhet region, the village farmers were dependent upon the buffaloes mostly for draught and meat purposes, being an integral part of the farming system in this area. It is economically important in this region and could be used as a "small tractor" for farmers and its milk and meat are nutritionally rich. Maximum day time the buffaloes were grazed in the pasture land harvested paddy fields, and along roadsides and fulfilled their physiological demands.

The gastrointestinal tract (GIT) of animals harbors a variety of parasites particularly helminths and ciliates which cause clinical and subclinical parasitism. Gastrointestinal parasitism is the most important disease encountered by the livestock sector of Bangladesh and is thought to be one of the major constraints in the development of dairy cattle worldwide [11, 12]. Gastrointestinal parasites not only affect the health of the animal but also affect the productive and reproductive performance, loss in body weight digestive disturbances, and emaciation for a longer period [13, 14]. Helminths are a primary factor in the reduced productivity of ruminants in many parts of the world, particularly in developing countries where nutrition and sanitation are generally poor [15]. The chronic insidious form of helminthiasis is much more common and is associated with greater economic losses than acute diseases and many other lethal infectious diseases [16].

Among the parasitic diseases, gastrointestinal parasites are such as *Bunostomum sp., Haemonchus spp., Trichostrongylus spp., Cooperia spp., Oesophagostomum spp., Trichuris spp.* and *Strongyloides spp., Fasciola sp., Paramphistomum sp*. are most common [17]. Anthelmintics are used traditionally as an integral part of helminthic control strategies for grazing livestock to prevent production losses from parasitic infections. The continuous and indiscriminate use of the same anthelmintics over years together as the sole means of control is now failing due to the emergence of resistant strains of helminths. Resistance to the commonly used anthelmintics in GI parasites of animals has become an increasingly widespread problem throughout the world [18]. Fairweather and Boray (1999) detected resistance to triclabendazole in the field and suggested minimizing the development of resistance including the use of synergistic drug combinations, together with the design of integrated management programs and the search for alternatives to drugs, in particular, vaccines [19]. There are different combined preparation of triclabendazole and levamisole throughout the world. In Bangladesh, only a combination of triclabendazole and levamisole is present and used as a broad-spectrum anthelmintic [20]. The anthelmintic activities and therapy of albendazole, levamisole, and ivermectin have been studied in buffalo [21]. Albendazole act by inhibiting tubulin polymerization and levamisole works as a nicotinic acetylcholine receptor agonist that causes continued stimulation of the parasitic worm muscles, leading to paralysis [22]. Tetramisole is a broad-spectrum anthelmintic that acts on both mature and immature stages of many important gastrointestinal nematodes and lungworms in cattle, sheep, goats, and pigs [23]. The advantage of tetramisole over other similar anthelmintics is that when used against one particular species of parasite, it at the same time kills other species, although their presence has not been manifested.

Oxyclozanide is a salicylanilide anthelmintic that acts by uncoupling oxidative phosphorylation, thereby decreasing the availability of high-energy phosphates such as ATP to the parasites; it is formulated as an oral drench containing oxyclozanide alone or in combination with levamisole hydrochloride [24]. Triclabendazole acts on the fumarate reductase enzyme and inhibits its function which is required for the conversion of fumarate to succinate [25]. Various drugs are found in the market which is used against GI parasites. But accuracy varies from drug to drug. Sometimes resistance is grown against the specific anthelmintics in the animal body. So, the present study is designed to know about the drug efficacy of different anthelmintics used in buffalos.

2. MATERIALS AND METHODS

The experiment was conducted in the laboratory of the Department of Medicine, Faculty of Veterinary and Animal Sciences, Sylhet Agricultural University, Sylhet, Bangladesh. In this experiment albendazole, levamisole, combined preparation of tetramisole + oxyclosanide, and levamisole + triclabendazole were used to evaluate their anthelmintic efficacy based on EPG count. The fecal egg count reduction test is widely used and the most accepted test to monitor anthelmintic efficacy test in the field. The design of the efficacy study of different anthelmintics in buffalo is shown in Fig. 1.

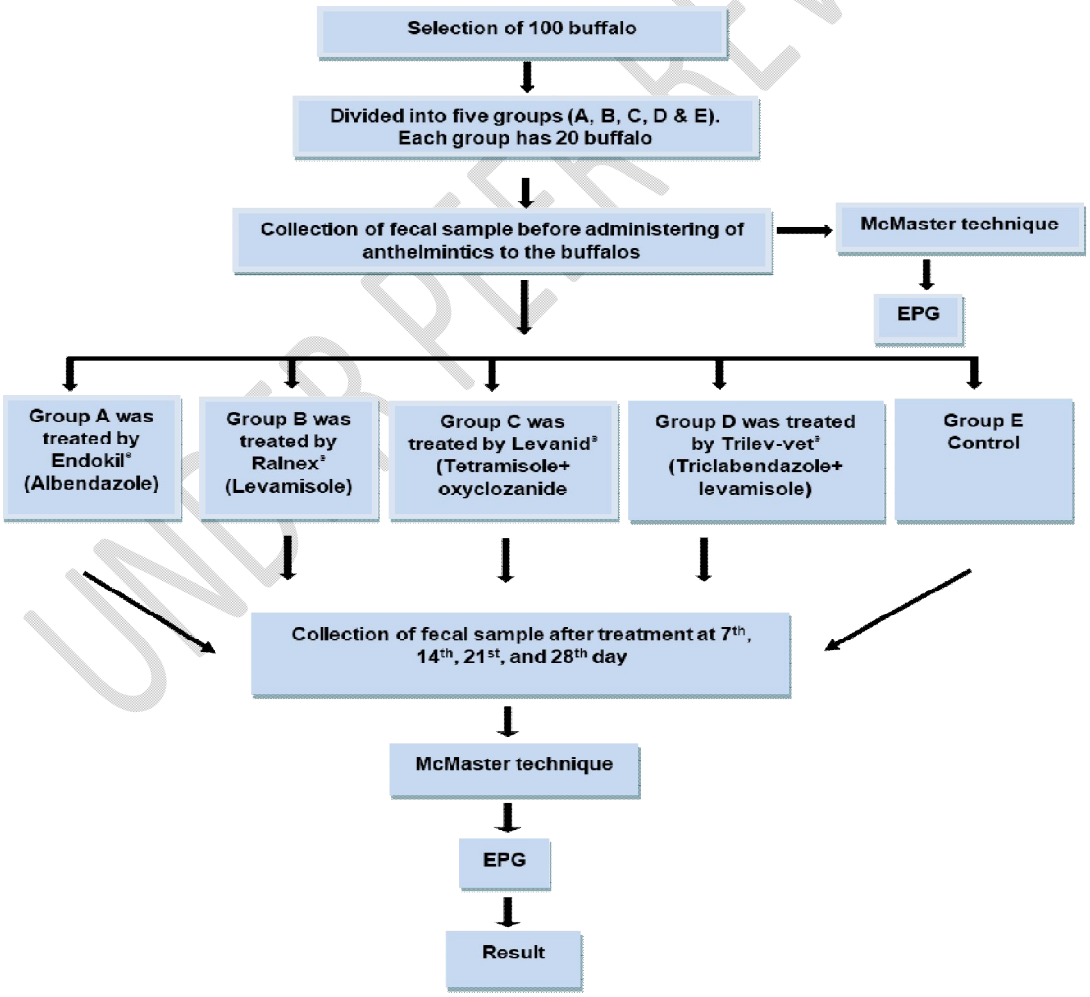

Fig. 1. Study design of the efficacy of different anthelmintics in buffalo.

2.1 SELECTION OF BUFFALO

A total of 100 buffalo were selected for this study which was suspected to suffer from single or mixed parasitic infestation. These 100 buffalo were divided into 5 equal groups (A, B, C, and D) each of which contained 20 buffalo. All these buffaloes were maintained under nearly identical conditions. They were kept in the door at night and part of the day. They were on average 3-5 years of age, detected by dentition [26]. Fecal samples were collected from 100 buffaloes and were examined to find out the eggs of parasites. EPG of feces was detected by the McMaster technique [3, 27].

Fig. 2. Swamp buffalos in Sylhet region of Bangladesh.

2.2 EXPERIMENTAL DRUGS

A total of 4 types of bolus of 4 different preparations were selected at first and then collected from the local market. Bolus, their composition, and the name of the producer company are given in table 1.

Table 1. Composition and name of the producer company of the bolus used in the experiment

SL. No.	Trade name	Composition	Company
1.	Endokil®	Albendazole	ACI animal health Ltd
2.	Ralnex®	Levamisole	Novartis Ltd
3.	Levanid®	Tetramisole+Oxyclozanide	The Acme laboratories
4.	Trilev-vet®	Triclabendazole+Levamisole	Square pharmaceuticals Ltd.

2.3 EXPERIMENTAL SCHEDULE

Among the 5 groups, one group (E) was kept as control other three groups were treated with 4 drugs as per the following schedule (Table 2).

Table 2. Drugs with dose and route of administration for Buffalo

Group	Trade Name	Trade Dose and route
A	Endokil®	1 bolus/80 kg body weight; PO
B	Ralnex®	1 bolus/41-75 body weight kg; PO

C	Levanid®	1 bolus/100-150 kg body weight; PO
D	Trilev-vet®	1 bolus/75 Kg body weight; PO
E (Control)	Null	Null

All 5 groups of buffalo were observed for 28 days after treatment and the following parameters were studied before treatment (0 days) and after treatment (7^{th}, 14^{th}, 21^{st}, and 28^{th} day) with 4 drugs.

2.4 COLLECTION OF FECAL SAMPLE

The fecal sample was collected directly from the rectum of each buffalo. The samples were numbered according to the neck belt number of the buffalo. In an extra sheet, the neck belt number of the buffalo, date of collection, age of the animal, health condition, and other particulars were recorded. Samples were transferred immediately after collection in sterile zipper bags to store in an ice box and then sending it to the laboratory for examination.

2.5 DATA ANALYSIS

The data generated were entered into SPSS (Statistical Package for Social Sciences) version 26 and Microsoft Excel (2013) Worksheet with the help of data analysis tools. Descriptive statistics were performed to calculate the mean and standard error. The one-way ANOVA was performed to evaluate the differences among the mean effect of 4 drugs. All the analyses were performed at the level of $P<0.05$.

3. RESULT

Fecal samples were collected from 100 buffalo and were examined to find out the eggs of gastrointestinal parasites. All the 100 buffalo were found to be affected with mixed parasitic infection at day 0 (Pretreatment period). These parasites were *Fasciola gigantica*, *Haemonchus spp., Strongyles spp.*, *Paramphistomum spp., Trichuris spp.,* and *Bunostomum spp.* After that 4 drugs namely Endokil® (ACI), Ralnex® (Novartis), Levanid® (Acme), Trilev-Vet® (Square) were applied to four groups containing 20 buffalo in each group. The result of the experiment is given in Table No. 3.

In group A, buffalos were suffering from different types of gastrointestinal parasites. The mean EPG count before treatment (0 days) was 478.57±37.57 but after treatment with Endokil®, the mean EPG count was reduced to 437.14±15.23, 385.00±8.86, 331.43±11.42 and 272.85±27.21 at day 7, 14, 21 and 28 respectively. In this group, the reduction of EPG count was 8.65%, 19.55%, 30.75%, and 42.99% on days 7, 14, 21, and 28 respectively.

In group B the mean EPG count before treatment (0 days) was 365.40±11.86 but after treatment with Ralnex®, the mean EPG count was reduced to 298.57±11.42, 215.71±20.08, 167.29±11.96 and 109.05±15.01 at day 7, 14, 21 and 28 respectively. In this group, the reduction of EPG count was 18.29%, 40.9654.22%, and 70.16% on days 7, 14, 21, and 28 respectively.

In group C the mean EPG count before treatment (0 days) was 494.21±28.61 but after treatment with Levanid® the mean EPG count was reduced to 328.57±15.40, 235.78±12.86, 122.43±23.23 and 92.86±8.12at day 7, 14, 21 and 28 respectively. In this group, the reduction of EPG count was 33.52 %, 52.29%, 75.23%, and 81.21% on days 7, 14, 21, and 28 respectively.

In group D the mean EPG count before treatment (0 days) was 594.13±32.10 but after treatment with Trilev-Vet®, the mean EPG count was reduced to 380.21±11.90, 255.78±19.61, 121.12±32.12 and 73.61±12.23 at day 7, 14, 21 and 28 respectively. In this group, the reduction of EPG count was 36.01%, 56.95%, 79.61%, and 87.61% on days 7, 14, 21, and 28 respectively.

In group E the mean EPG count at day 0 was 315.12±22.85 but the mean EPG count increased to 417.14±39.91, 465.12±17.12, 510.14±31.46 and 575.32±12.92 at day 7, 14, 21 and 28 respectively. In this group, the increase in EPG count was 32.38%, 47.60%, 61.89%, and 82.57% on days 7, 14, 21, and 28 respectively.

Table 3. Comparative efficacy of modern anthelmintics against gastro-intestinal helminthiasis in Buffalo

Group	Pre-treatment (EPG)	Post-treatment (EPG)							
	Day 0 (Mean±SE)	Day 7 (Mean±SE)	% of reduction in 7th days	Day 14 (Mean±SE)	% of reduction in 14th days	Day 21 (Mean±SE)	% of reduction in 21st days	Day 28 (Mean±SE)	% of reduction in 28th days
A Endokil®	478.57±37.57	437.14±15.23	8.65	385.00±8.86	19.55	331.43±11.42	30.75	272.85±27.21	42.99
B Ralnex®	365.40±11.86	298.57±11.42	18.29	215.71±20.08	40.96	167.29±11.96	54.22	109.05±15.01	70.16
C Levanid®	494.21±28.61	328.57±15.40	33.52	235.78±12.86	52.29	122.43±23.23	75.23	92.86±8.12	81.21
D Trilev-vet®	594.13±32.10	380.21±11.90	36.01	255.78±19.61	56.95	121.12±32.12	79.61	73.61±12.23	87.61
E (Control)	315.12±22.85	417.14±39.91	32.38 (increase)	465.12±17.12	47.60 (increase)	510.14±31.46	61.89 (increase)	575.32±12.92	82.57 (increase)

The mean value of EPG obtained on the 28th day by administration of 4 different drugs is compared. The obtained P-value was 0.002, where the significance level was 0.05.

4. DISCUSSION

Fecal egg count tests were also conducted by different scientists to evaluate the efficacy of different drugs [28, 29]. *Fasciola* spp., *Haemonchus* spp., *Strogyles* spp., *Paramphtstnmum* spp., and *Trichuris* spp. *Bunostomum* sp. was detected during the pre-treatment study which was also observed by others [30, 31]. Moreover geographical and topographical condition favors the growth and multiplication of parasites in buffalo. So the percentage of mixed parasitic infection in buffalo in Bangladesh is very high.

Among the four groups of drugs applied, the efficacy is found best in the case of Trilev-vet® and comparatively poor in the case of Endokil® (albendazole). Trilev-vet® (triclabendazole+levamisole) has reduced the mean EPG count at 36.81%, 56.95%, 79.61%, and 87.61% at day 7, 14, 21, and 28 respectively in the D group while Endokil® reduced the mean EPG count at 8.65%, 19.55%, 30.75% and 42.99% at day 7, 14, 21 and 28 respectively in A group. These findings were almost similar to the findings of some others [32, 33, 34]. After comparing the mean value of EPG on the 28th day by administration of four different drugs the P-value was found 0.002, where the significance level was 0.05. It indicated that the mean of the four drugs was dissimilar in efficacy and thereby the Trilev-Vet® effects better than the other three drugs.

In contrast with Endokil®, Levanid® (tetramisole+oxyclozanide) was performing better as 81.21% reduction of the EPG count on 28th days. Higher rate of therapeutic efficacy in case of tetramisole and oxyclozanide treatment were observed by others [35, 36]. The efficacy of albendazole and levamisole individually against helminth parasites is encouraging but variable this findings of the present study were similar to the findings of some other authors [37, 38]. However, the individual treatment either with triclabendazole or levamisole sometimes becomes resistant [28, 39]. So, now a day to overcome the development of resistance and for the synergistic effect, the combined preparation is well practiced by many authors [20, 40]. In the present study combined preparation with triclabendazole and levamisole was found to be highly encouraging. The difference in efficacy may be due to anthelmintic resistance, compositional, and quality variation [33].

From the above-stated findings, it is evident that the control program of mixed parasitic infection should be given priority. There are so many difficulties in controlling mixed parasitic infection other than the use of anthelmintics. But anthelmintics can be used as a good prophylactic agent. So the determination of the most efficient drugs against mixed parasitic infection is essential. In this study, the most commonly used drugs were selected and their efficacy against mixed natural parasitic infection in buffalo was determined.

5. CONCLUSION

Among the four groups, the highest efficacy was found in the case of Trilev-vet® (levamisole + triclabendazole) which reduced EPG by 87.61%, and the lowest efficacy was found in the case of Endokil® (albendazole) which reduced the EPG 42.99% in 28th day. From the findings, we can conclude that synergistic actions of levamisole and triclabendazole combinations reduced the EPG count and were very much helpful in buffalo parasitic treatment.

REFERENCES

1. Al-Jamass RK. Determination of some featural and production traits on Iraqi water buffalo in Badosh. Buffalo Newsletter. 1999;12:12-3.

2. Hamid MA, Ahmed S, Rahman MA, Hossain KM. Status of buffalo production in Bangladesh compared to SAARC countries. Asian J Animal Sci. 2016;10(6):313-29.

3. Solusby EJL. Heilminths, Arthropods and Protozoa of Domesticated Animals, 7th ed. Philadelphia, Bailliere Tindall, London. 1986:3-4.

4. El-Sherif AM, Abdel-Gawad MA, Lotfy HS, Shokier KA. Impact of gastrointestinal nematodes and some enteric protozoal affections on the health of buffalo-calves. Egypt J Agri Res. 2000;78(1, Special Issue):127-35.

5. Department of Livestock Services (DLS). Livestock Economy at a glance 2021-2022. Accessed 5 December 2022. Available: http://www.dls.gov.bd/site/page/22b1143b-9323-44f8-bfd8-647087828c9b/Livestock-Economy

6. Köhler-Rollefson I, Rathore H, Mathias E. Local breeds, livelihoods and livestock keepers' rights in South Asia. Trop Ani Health Prod. 2009;41(7):1061-70.

7. Cockrill WR. Present and future of buffalo production in the world. In Proceedings of the Fifth World Buffalo Congress; 1994; Sao Paulo, Brazil 1994.

8. FAO. Production Year Book. 2009. Vol. 51.pp-56.

9. Bangladesh Bureau of Statistics (BBS). 2021. Accessed 5 December 2022. Available:http://bbs.portal.gov.bd/sites/default/files/files/bbs.portal.gov.bd/page/5464ccb8_f7a2_4ad2_a1ef_2a44daa0af6f/2022-03-29-06-10-2ed46e9d1b5f9613093b659f51364ca3.pdf

10. Faruque MO, Hasnath MA, Siddique NN. Present status of buffaloes and their productivity in Bangladesh. Asian-Australasian J Animal Sci. 1990;3(4):287-92.

11. Regassa F, Sori T, Dhuguma R, Kiros Y. Epidemiology of gastrointestinal parasites of ruminants in Western Oromia, Ethiopia. Int J Applied Res Vet Medi. 2006;4(1):51.

12. Kakar MN, Kakarsulemankhel JK. Prevalence of endo (trematodes) and ecto-parasites in cows and buffaloes of Quetta, Pakistan. Pakistan Vet J. 2008;28(1):34.

13. Radostits, OM., Gay, GC, Blood, DC and Hinchkiliff, KW. Veterinary Medicine 9th ed. EIBS and Bailliere Tindal. 2000.

14. Rahman MM, Samad MA. Prevalence of subclinical gastro-intestinal parasitosis and their effects on milk production with therapeutic management in red chittagong cattle. Bangl J Vet Med. 2010;8(1):11-16.

15. Pralomkarn W, Kochapakdee S, Saithanoo S, Choldumrongkul S. Effect of supplementation and internal parasites on growth of cross-bred goats under village environments in southern Thailand. Thai J Agri Sci. 1994.

16. Kumsa B, Tolera A, Nurfeta A. Comparative efficacy of seven brands of albendazole against naturally acquired gastrointestinal nematodes in sheep in Hawassa, southern Ethiopia. Tur J Vet & Ani Sci. 2010;34(5):417-25.

17. Rahman MH and Mondal MMH. Helminth parasites of cattle (*Bos indicus*) in

Bangladesh. Ind J Parasitol. 1983;7:173-174.

18. Meenakshisundaram A, Anna T and Harikrishnan J. Prevalence of drug-resistant GI nematodes in an organized sheep farm. Vet World. 2014;7(12): 1113-1116.

19. Fairweather I, Boray JC. Fasciolicides: efficacy, actions, resistance and its management. The Vet J. 1999;158(2):81-112.

20. Islam MR and Islam MM. Veterinary Prescription Guide 04. Nokta Publications. 69/G, Green road, Panthapath, Dhaka, pp: 39-44. 2004.

21. Kasarilikar VR, Kumar SP, Udupa KG, Shivaparkash BV, Patial NA, Mutnal AB. Comparative efficacy of levamisole against ascariasis in buffaloe calves. Ind Vet J. 1997;76:150-152.

22. Loukas A, Hotez PJ. Chemotherapy of helminth infections. Goodman and Gilman's The pharmacological basis of therapeutics, 11th Edition. New York: McGraw-Hill. 2006:1073-93.

23. Malik MA, Sajid MS, Abbas RZ, Aleem MT, Anjum FR, Khan A et al. Anthelmintic Drug Resistance in Livestock: Current Understanding and Future Trends. 2022.

24. Einstein R, Jones RS, Knifton A, Starmer GA. Principles of veterinary therapeutics. Longman Scientific and Technical; 1994.

25. Bennett JL, Thompson DP. Mode of action of antitrematodal agents. In Chemotherapy of parasitic diseases 1986 (pp. 427-443). Springer, Boston, MA.

26. Banerjee GC. A textbook of animal husbandry. 1988. 8th ed. Oxford & IBII Publishing Co.

27. Gordong HMcL and Whitlock HV. A new technique for counting nematode eggs in sheep feces. JCSIR, Australia, 1939;12:50-52.

28. Chandrawathani P, Adnan M, Waller PJ. Anthelmintic resistance in sheep and goat farms on Peninsular Malaysia. Vet Paras. 1999;82(4):305-10.

29. Coles GC, Jackson F, Pomroy WE, Prichard RK, von Samson-Himmelstjerna G, Silvestre A et al. The detection of anthelmintic resistance in nematodes of veterinary importance. Vete paras. 2006;136(3-4):167-85.

30. Haq S, Shaikh H. A survey of helminths parasitising the gastro-intestinal tracts of goats and sheep in East Pakistan. Pak J Soil Sci. 1968;2(2):54-62.

31. Hoque MS, Samad MA. Prevalence of clinical diseases in dairy cross-bred cows and calves in the urban areas in Dhaka. Bangla Vet J. 1996;30(3-4):118-29.

32. Hassan M, Hoque MA, Islam SA, Khan SA, Hossain MB, Banu Q. Efficacy of anthelmintics against parasitic infections and their treatment effect on the production and blood indices in Black Bengal goats in Bangladesh. Tur J Vet & Ani Sci. 2012;36(4):400-8.

33. Khair A, Uddin MB, Hossain MM, Mamun MA, ISLAM M. Efficacy of different

anthelmintic preparations against gastrointestinal parasitic infestation in sheep. Int J Sustain Agril Tech. 2010;6(10):08-12.

34. Islam N, Awal MA, Islam MS, Sobhan MA, Rahman MM. Efficacy of levamisole and triclabendazole against gastrointestinal nematodes and trematodes in crossbred cows. Ind J Vet Med. 2005;25(1):24.

35. Reid JF, Armour J, Jennings FW, Urquhart GM. Efficacy of tetramisole. Studies on the efficacy of tetramisole in the treatment of Pre-Type II and Type II bovine ostertagiasis. Vet Rec. 1968;83(1):14-6.

36. García-Dios D, Díaz P, Viña M, Remesar S, Prieto A, López-Lorenzo G, Cao JM, Panadero R, Díez-Baños P, López CM. Efficacy of oxyclozanide and closantel against rumen flukes (Paramphistomidae) in naturally infected sheep. Animals. 2020;10(11):1943.

37. Wrigley J, McArthur M, McKenna PB, Mariadass B. Resistance to a triple combination of broad-spectrum anthelmintics in naturally-acquired *Ostertagia circumcincta* infections in sheep. New Zea Vet J. 2006;54(1):47-9.

38. Biswas H, Roy BC, Hasan MM, Ahmed N, Dutta PK, Begum N, Talukder M. Efficacy of clinically used anthelmintics against toxocariasis of buffalo calves in Bangladesh. J Paras Dis. 2022;46(4):988-97.

39. Maingi N, Otieno RO, Weda EH, Gichohi VM. Effects of three anthelmintic treatment regimes against Fasciola and nematodes on the performance of ewes and lambs on pasture in the highlands of Kenya. Vet Res Communi. 2002;26(7):543-52.

40. Ibarra-Velarde F, Vera-Montenegro Y, Nájera-Fuentes R, Sánchez-Albarran A. Efficacy of combined chemotherapy against gastrointestinal nematodes and *Fasciola hepatica* in cattle. Vet Paras. 2001;99(3):199-204.