Chapter 1: Introduction

The food-borne trematodes *Fasciola gigantica* are the causative agents of fascioliasis (fasciolosis). Although, *Fasciola hepatica* is not found in Bangladesh due to absent of intermediate host, *Fasciola gigantica* is very common and that is responsible of causing fascioliasis. *Fasciola gigantica* parasitizes a wide spectrum of domestic and wild animals (e.g., sheep, cattle, rats, and deer), and it causes a huge economic loss annually to the agriculture sector worldwide through losses of milk and meat yields (Mas-coma *et al*., 2005; and Robinson *et al*., 2009).

Bovine fasciolosis usually lacks visible clinical signs. However, the cattle infection was chronic which it causes weight loss, reduction in the milk yield and fertility problems (Genicot *et al*., 1991; Schweizer *et al*., 2005; Charlier *et al*., 2007 and Loyacano *et al*., 2002). Liver cirrhosis was also determined (Marcos *et al*., 2007). Vercruysse and Claerebout (2001) reported that significant production losses occur in *Fasciola gigantica* infection.

In addition, an estimated 90 million people are at risk of fascioliasis and up to 17 million individuals are infected (Keiser *et al*., 2009).

Basically, fascioliasis control in ruminants depends on the administration of anthelmintics. The flukicides are effectively used both against immature and adult flukes. Administration of these flukicides at certain times of the year causes destruction of the parasite life cycle achieving successfully control of bovine fasciolosis (Richards *et al*., 1990; Parr and Gray, 2000; Mezo *et al*., 2008). However, since the variety of flukicides are very limited and animals are usually treated year after year without any monitoring of the efficiency of treatment, flukicide-resistant parasite populations may arise, making the control of fasciolosis difficult.

Due to its excellent safety profile and the high activity against both juvenile and adult liver flukes, triclabendazole (Fasinex®) and nitroxynil (Nitronex®) is the drug of choice for the treatment of human and veterinary fascioliasis. It is worrying however, that triclabendazole and nitroxynil resistance has been documented in Bangladesh due to indiscriminately using of those drugs.

Furthermore, *F. gigantica* strains resistant to three alternative fasciocidal drugs (rafoxanide, closantel, and luxabendazole) have been isolated (Fairweather *et al*., 1999). Due to the rapid spread of resistance, the small arsenal of fasciocidal drugs, and the absence of effective vaccines in field conditions, there is a pressing need for the discovery and development of novel drugs or drug combinations (McManus *et al*., 2006).

The use of drug combinations is an excellent strategy to avoid or delay drug resistance, since different drug targets are attacked simultaneously. Furthermore, drug combinations often are characterized by an increased activity and tolerability compared to that of monotherapy. Therefore, drug combinations are widely used in the treatment of infectious diseases (e.g., malaria, HIV, and tuberculosis), cancer, and chronic disorders (e.g., cardiovascular disease, diabetes, and pain management) (DeFronzo, R.A. 2016; Edwards *et al*., 2004; Gupta *et al*., 2010; Raffa *et al*., 2003; and Zhang, Y. 2007).

In my experiment, I used triclabendazole and nitroxynil against *Fasciola gigantica* to observe efficacy of the drugs.

The aims of the study are:

1. To observe the effect of triclabendazole and nitroxynil against *Fasciola gigantica* of cattle.
2. To determine the any resistance of those drugs against Fasciola.
3. To observe the dose dependent effect of Fasciola at various concentration at different time interval during in vitro drug trail.

Chapter 2: Materials and Methods

Sample collection

Live *Fasciola gigantica* was collected from an infected cattle liver that was bought from Riajuddin bazar at very early in the morning. The weight of the liver was approximately 13 kg. The liver then brought to our PRTC lab in order to collection of sample (live fasciola). After that, the live *Fasciola gigantica* were collected rapidly from the liver. Total 30 *Fasciola gigantica* were recovered from the liver.

Drugs

Triclabendazole (Fasinex®) and nitroxynil (Nitronex®) were bought from the market. For the *in vitro* experiments, stock solutions (3 and 10 mg/ml) were prepared in 100% (vol/vol) distilled water.

Culture medium

The specific culture medium for Fasciola is RPMI 1640 culture medium. The medium was collected from Genetics lab (CVASU).

Incubator

Normally Fasciola require 5% CO_2 during incubation. So I incubated the fasciola at 5% CO_2 at Poultry Research and Training Centre lab, CVASU.

In vitro drug assays

Adult *Fasciola giantica* flukes were recovered from the bile duct of liver of cattle and incubated for 1 h in RPMI 1640 culture medium at 37°C in an atmosphere of 5% CO_2. Adult worms were placed in three wells petridish containing 6 ml RPMI1640 and supplemented with 1% (vol/vol) antibiotics (50 µg/ml streptomycin and 50 IU/ml penicillin). Adult *Fasciola gigantica* were incubated in the presence of both triclabendazole (15µg/ml and 25µg/ml) and nitroxynil (15 µg/ml and 25µg/ml) for some period of time. The effects of single treatment of triclabendazole and nitroxynil at different concentration were studied. Six flukes were examined per drug and total twenty four flukes were exposed with the drugs. Six control flukes, without drug exposure, were included in each experimental set. The control well contained the normal saline and the flukes but no drug. The viability of adult flukes was examined at different time interval. They were examined after 12 hours, 24 hours, 36 hours, 48 hours, 60 hours and 72 hours interval under the low power magnification.

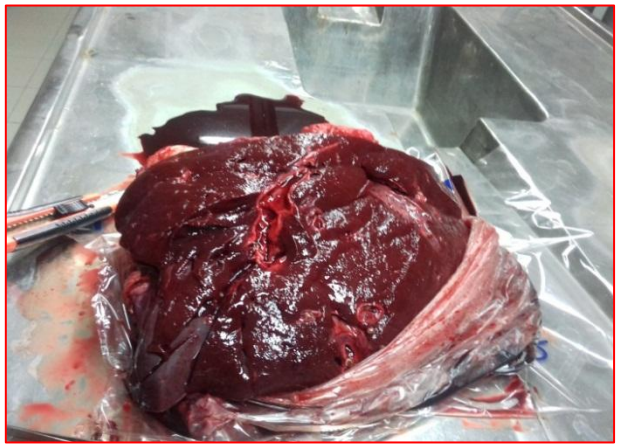

Fig 2.1: Liver of cattle

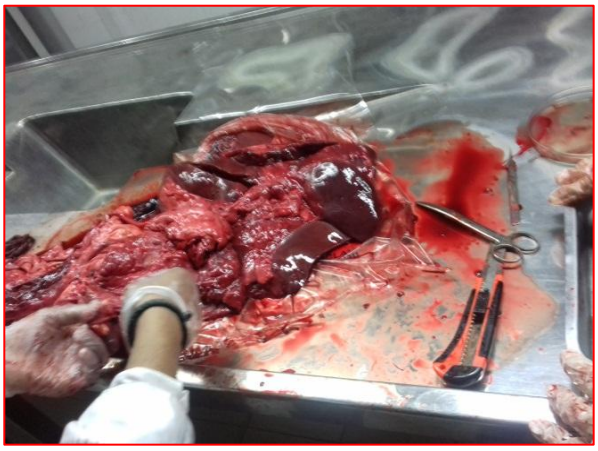

Fig 2.2: Collection of sample from Liver

Fig 2.3: collecting *Fasciola gigantica*

Fig 2.4: *Fasciola gigantica* without drug

Fig 2.5: Triclabendazole

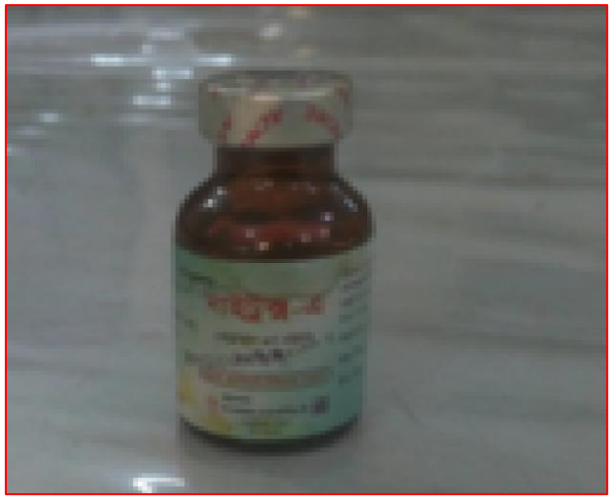

Fig 2.6: Nitroxynil

Fig 2.7: Preparation of Drugs

Fig 2.8: Culture medium for Fasciola

Fig 2.9: Incubator

Fig 2.10: Incubation of Fasciola with drugs

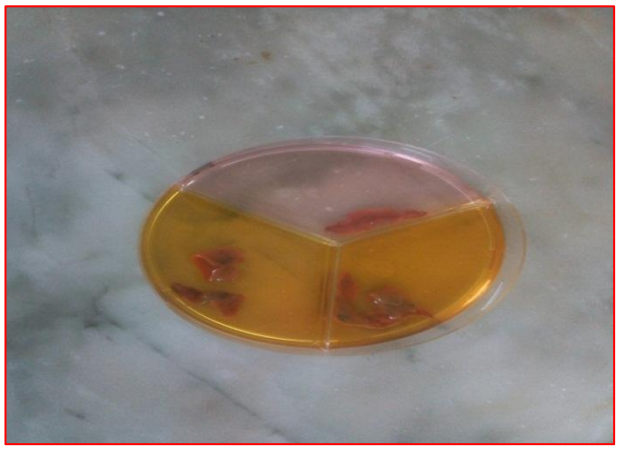

Fig 2.11: Observation of samples

Chapter 3: RESULTS

Table 1: Observation of viability of Fasciola treated with Triclabendazole at different time interval at different concentration of drug.

Observation Time(Hour)	Amount of drug given			
	15µg/ml		25µg/ml	
	Live	Dead	Live	Dead
12 hour	6	0	6	0
24 hour	6	0	5	1
36 hour	5	1	4	2
48 hour	2	4	1	5
60 hour	0	6	0	6

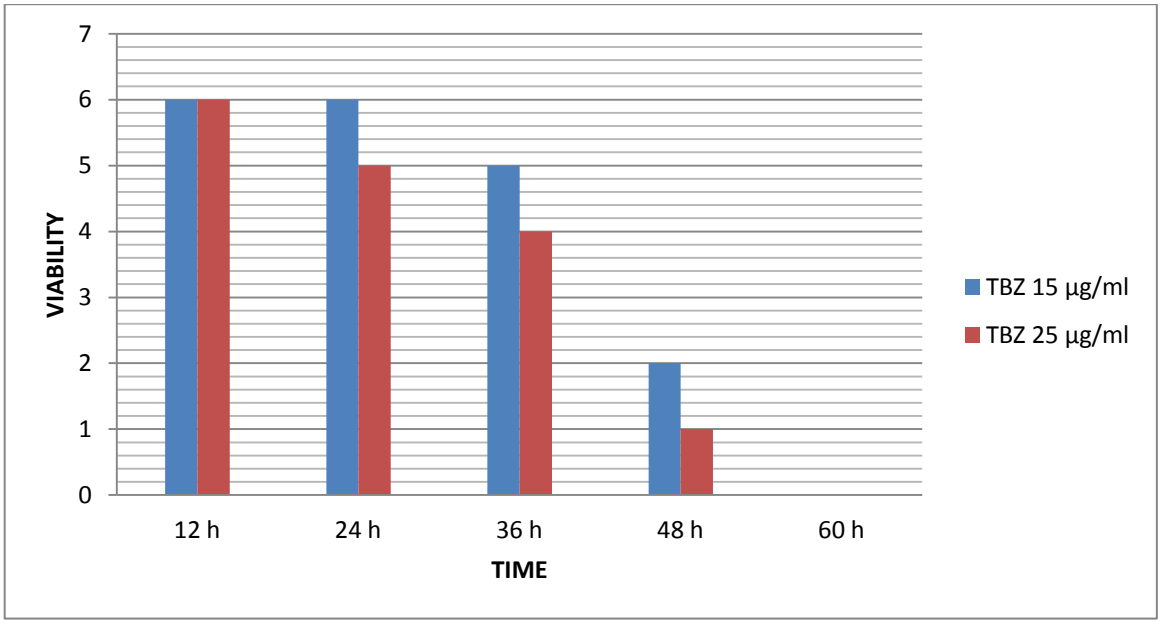

Fig 3.1: viability of Fasciola under different concentration of Triclabendazole.

Table 2: Observation of viability of Fasciola treated with Nitroxynil at different time interval at different concentration of drug.

Observation Time(Hour)	Amount of drug given			
	15µg/ml		25µg/ml	
	Live	Dead	Live	Dead
12 hour	6	0	6	0
24 hour	6	0	5	1
36 hour	5	1	4	2
48 hour	3	3	2	4
60 hour	2	4	0	6
72 hour	0	6	0	6

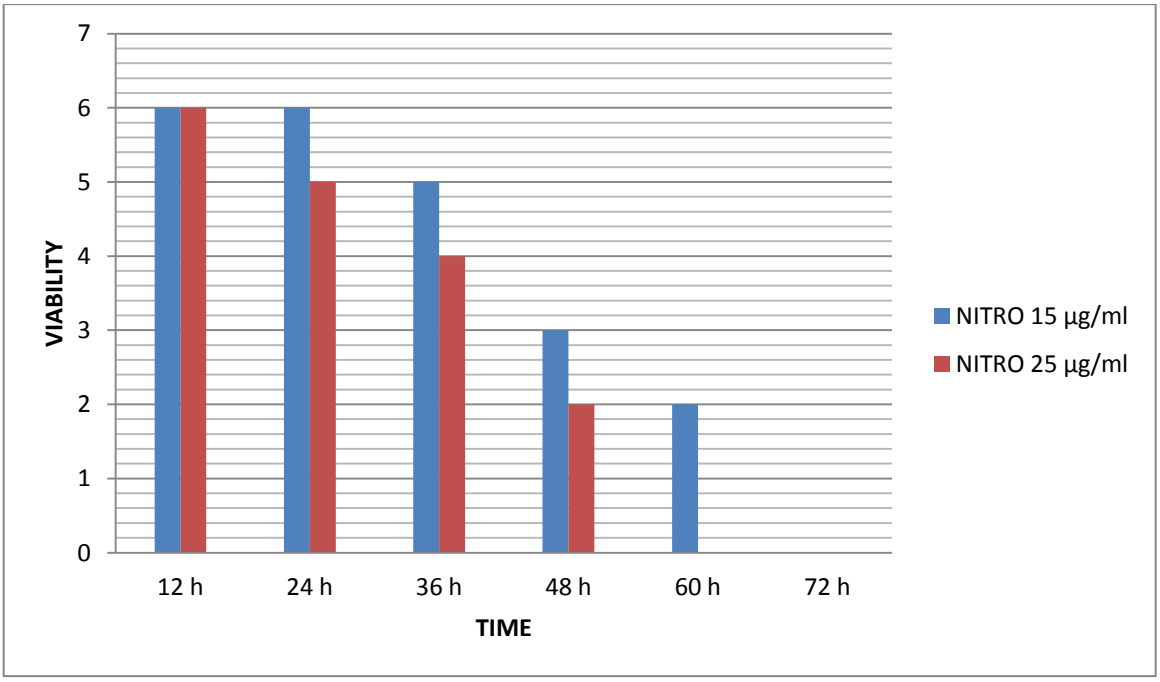

Fig 3.2: viability of Fasciola under different concentration of Nitroxynil.

Temporal drug effects (monotherapy) on the viability on adult *F. gigantica* in vitro are presented in Fig 1. Adult *F. gigantica* controls (*n*=6) showed normal activities during the entire observation period. Flukes incubated in the presence of triclabendazole showed a fast decrease of movements, and after 48 hours most of the worms had died. Differences in the effect of the triclabendazole concentration on *F. gigantica* were observed. Increased dose of triclabendazole lead to more sensitive of the drug to Fasciola. Dose of triclabendazole 25 µg/ml showed more potent than 15µg/ml. Lower mortality rates were found at 15µg/ml of triclabendazole than 25 µg/ml. *F. gigantica* exposed to triclabendazole at 25 µg/ml showed a rapid decrease in viability, and after 48 hours only minimal activities were recorded.

On the other hand, drug effects of nitroxynil on the viability on adult *F. gigantica* in vitro are presented in Fig 2. Again there were six control flukes were used. Adult *F. gigantica* controls (*n*=6) showed normal activities during the entire observation period. Flukes incubated in the presence of nitroxynil showed a late decrease of movements, and after 60 hours most of the worms had died. Differences in the effect of the nitroxynil concentration on *F. gigantica* were observed also. Increased dose of nitroxynil lead to more sensitive of the drug to fasciola. Dose of nitroxynil 25 µg/ml showed more potent than 15µg/ml. Delayed mortality rates were found at 15µg/ml of nitroxynil than 25 µg/ml. *F. gigantica* exposed to nitroxynil at 25 µg/ml showed a decrease in viability, and after 60 hours only minimal activities were recorded.

Chapter 4: Discussion

Cases of triclabendazole and nitroxynil resistance are continuously documented from livestock and hence new strategies for the treatment of *F. gigantica* infections are needed (Fairweather, I. 2009). Today, drug combinations are a popular tool to decrease the selection pressure and increase parasitological cure rates to reduce toxicity (Fairweather *et al.*, 1999).

In addition, drug combinations are less likely to produce adverse effect as reduced dosages are used. The present study investigates the monotherapy of triclabendazole and nitroxynil to the adult fasciola where both of the drugs are sensitive to fasciola. Triclabendazole showed more sensitive to fasciola than niroxynil. This is shown in the following graph:

Table 1 and Table 2; Graph 3.1 and 3.2-

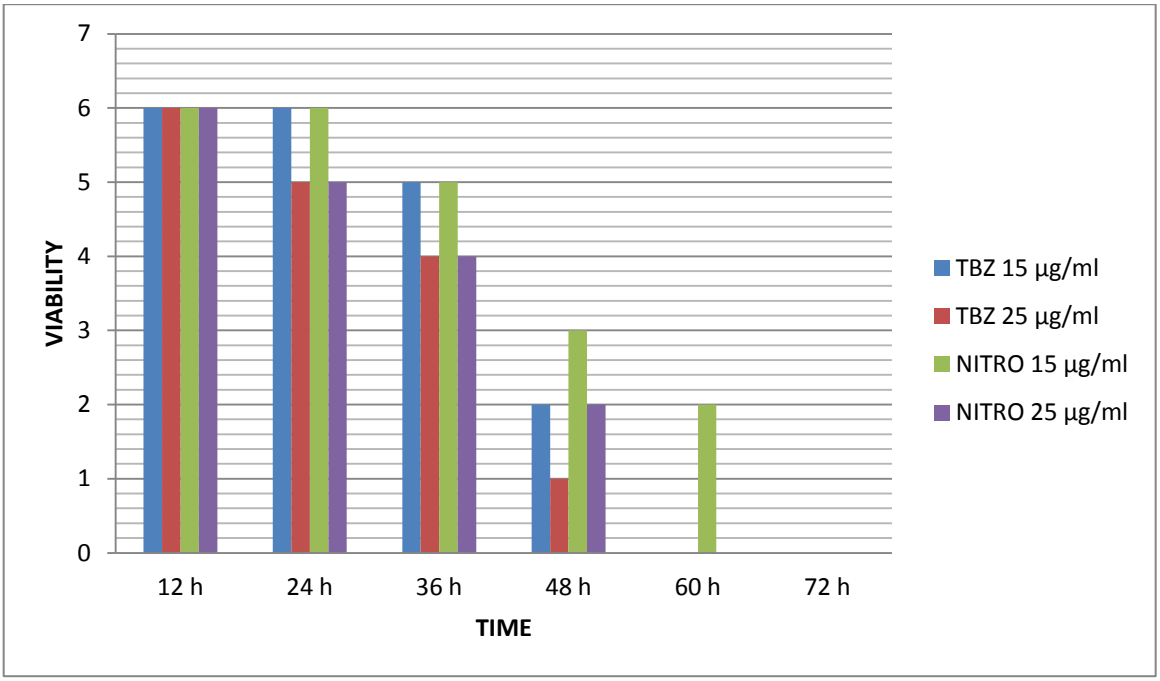

Fig 4.1: Dose dependent effect of triclabendazole and nitroxynil on sensitivity of *Fasciola gigantica* at various concentrations.

So, the use triclabendazole is more effective than nitroxynil against Fasciola. The dosage of triclabendazole between 5 mg/kg and 7.5 mg/kg are very effective against fasciola. But 7.5 mg/kg body weight is more effective and reduces the Fasciola burden at minimum level. Besides, nitroxyil 7.5 mg/kg body weight also effective against Fasciola. Furthermore, while our in vitro study was based on periodic phenotypic evaluation, more sophisticated approaches such as calorimetric measurement (Manneck *et al*., 2010), which continuously measures the energy release of the worms, are required to evaluate drug-worm interactions in more detail.

I did not use dosages higher than 7. 5 mg/kg in my studies, since a 10 mg/kg body weight of those drugs's dose has been shown to achieve worm burden reductions of 85 to 100% (Keiser *et al*., 2005; and unpublished observations). Nonetheless, it would be interesting to evaluate intermediate triclabendazole and nitroxynil doses. It has been demonstrated that triclabendazole affects spermatogenic and vitelline cells of *F. gigantica* and in vitro (Diab *et al*., 2010; Stitt *et al*., 1996 and Stitt *et al*., 1992).

Limitations

I have following limitations in my study:

- The number of flukes in my investigation was small.
- In vivo drug trail also necessary to determine the efficacy of those drugs.
- Calorimetric measurement measures the energy release of the worms are required to evaluate drug-worm interactions in more detail. But I can not use it.

Conclusion

I confirmed the promising fasciocidal properties of Triclabendazole and Nitroxynil in my study. However, triclabendazole showed more effectiveness than nitroxynil. The dose variation also included in my study. Besides, the sensitivity of the drugs increases when the doses have increased. So saying that, my results indicate that, triclabendazole and nitroxynil is highly effective drug for the treatment of Fasciola species infection in animals.

References

Bennett, J. L., and P. Kohler. 1987. Fasciola hepatica: action in vitro of triclabendazole on immature and adult stages. Exp. Parasitol. 63:49–57.

Boray, J. C., P. D. Crowfoot, M. B. Strong, J. R. Allison, M. Schellenbaum, M. Von Orelli, and G. Sarasin. 1983. Treatment of immature and mature Fasciola gigantica infections in sheep with triclabendazole. Vet. Rec. 113: 315–317.

Charlier J, Duchateau L, Claerebout E, Williams D, Vercruysse J. Associations between anti-Fasciola hepatica antibody levels in bulk-tank milk samples and production parameters in dairy herds. Preventive Veterinary Medicine 2007;78:57-66.

DeFronzo, R. A. 2010. Current issues in the treatment of type 2 diabetes. Overview of newer agents: where treatment is going. Am. J. Med. 123:S38– S48.

Diab, T. M., H. H. Mansour, and S. S. Mahmoud. 2010. Fasciola gigantica: parasitological and scanning electron microscopy study of the in vitro effects of ivermectin and/or artemether. Exp. Parasitol. 124:279–284.

Edwards, G., and S. Krishna. 2004. Pharmacokinetic and pharmacodynamics issues in the treatment of parasitic infections. Eur. J. Clin. Microbiol. Infect. Dis. 23:233–242.

Fairweather, I. 2009. Triclabendazole and nitroxynil progress report, 2005–2009: an advancement of learning J. Helminthol. 83:139–150.

Fairweather, I., and J. C. Boray. 1999. Fasciolicides: efficacy, actions, resistance and its management. Vet. J. 158:81–112.

Gupta, A. K., S. Arshad, and N. R. Poulter. 2010. Compliance, safety, and effectiveness of fixed-dose combinations of antihypertensive agents: a metaanalysis. Hypertension 55:399–407.

Genicot B, Mouligneau F, Lekeus P. Economic and production consequences of liver fluke disease in double-muscled fattening cattle. Journal of Veterinary Medicine B 1991;38:203-8.

Hoey, H. Fletcher, and I. Fairweather. 2004. Response of two isolates of Fasciola hepatica to treatment with triclabendazole in vivo and in vitro. Parasitol. Res. 94:427–438.

Keiser, J. 2010. In vitro and in vivo trematode models for chemotherapeutic studies. Parasitology 137:589–603.

Keiser, J., D. Engels, G. Buscher, and J. Utzinger. 2005. Triclabendazole for the treatment of fascioliasis and paragonimiasis. Expert Opin. Investig. Drugs 14:1513–1526.

Keiser, J., and J. Utzinger. 2009. Food-borne trematodiases. Clin. Microbiol. Rev. 22:466 483.

Loyacano AF, Williams JC, Gurie J, de Rosa AA. Effect of gastrointestinal nematodes and liver fluke on weight gain and reproductive performance of beef heifers. Veterinary Parasitology 2002;107:227-34.

Mas-Coma, S., M. D. Bargues, and M. A. Valero. 2005. Fascioliasis and other plant borne trematode zoonoses. Int. J. Parasitol. 35:1255–1278.

Marcos LA, Yi P, Machicado A, Andrade R, Samalvides S, Sa´nchez J, et al. Hepatic fibrosis and Fasciola hepatica infection in cattle. Journal of Helminthology 2007;81:381-6.

McManus, D. P., and J. P. Dalton. 2006. Vaccines against the zoonotic trematodes Schistosoma japonicum, Fasciola hepatica and Fasciola gigantica. Parasitology 133(Suppl.):S43–S61.

Mezo M, Gonza´lez-Warletam M, Castro-Hermida JA, Florencio MU. Evaluation of the flukicide treatment policy for dairy cattle in Galicia (NW Spain). Veterinary Parasitology 2008;157:235-43.

Manneck, T., O. Braissant, W. Ellis, and J. Keiser. 2010. Schistosoma mansoni: antischistosomal activity of the four optical isomers and the two racemates of

mefloquine on schistosomula and adult worms in vitro and in vivo. Exp. Parasitol. doi:10.1016/j.exppara.2010.08.011.

Parr SL, Gray JS. A strategic dosing scheme for the control of fasciolosis in cattle and sheep in Ireland. Veterinary Parasitology 2000;88:187-97.

Raffa, R. B., R. Clark-Vetri, R. J. Tallarida, and A. I. Wertheimer. 2003. Combination strategies for pain management. Expert Opin. Pharmacother. 4:1697–1708.

Robinson, M. W., and J. P. Dalton. 2009. Zoonotic helminth infections with particular emphasis on fasciolosis and other trematodiases. Philos. Trans. R. Soc. Lond. B Biol. Sci. 364:2763–2776.

Richards RJ, Bowen FL, Essenwein RF, Steiger RF, Bu¨ scher G. The efficacy of triclabendazole and other anthelmintics against Fasciola hepatica in controlled studies in cattle. Veterinary Record 1990;126:213-6.

Smeal, M. G., and C. A. Hall. 1983. The activity of triclabendazole against immature and adult Fasciola gigantica infections in sheep. Aust. Vet. J. 60:329–331.

Stitt, A. W., and I. Fairweather. 1996. Fasciola hepatica: disruption of the vitelline cells in vitro by the sulphoxide metabolite of triclabendazole. Parasitol. Res. 82:333–339.

Stitt, A. W., and I. Fairweather. 1992. Spermatogenesis in Fasciola hepatica- an ultrastructural comparison of the effects of the anthelmintic, triclabendazole (fasinex) and the microtubule inhibitor, tubulozole. Invertebr. Reprod. Dev. 22:139–150.

Schweizer G, Braun U, Deplazes P, Torgerson PR. Estimating the financial losses due to bovine fasciolosis in Switzerland. Veterinary Record 2005;157:188-93.

Vercruysse J, Claerebout E. Treatment vs non-treatment of helminth infections in cattle defining the threshold. Veterinary Parasitology 2001;98:195-214.

Walker, S. M., B. McKinstry, J. C. Boray, G. P. Brennan, A. Trudgett, E. M.

Zhang, Y. 2007. Advances in the treatment of tuberculosis. Clin. Pharmacol. Ther. 82:595–600.

Acknowledgements

The author wishes to acknowledge the immeasurable grace and profound kindness of Almighty **"GOD"** the supreme authority and supreme ruler of universe, who empowers the author to complete the work successfully.

The author is ever grateful to **Professor Dr. Md. Ahasanul Hoque,** Honorable Dean, Faculty of Veterinary Medicine, Chittagong Veterinary and Animal Sciences University and **Professor Dr. A.K.M. Saifuddin**, Director, External Affairs, Chittagong Veterinary and Animal Sciences University, for arranging such type of research work as a compulsory part this internship programme and providing kind support in this regard.

The author feels proud in expressing his deep sense of great gratitude and indebtedness to respected teacher and supervisor Prof. **Dr. Md. Mohammad Alamgir Hossain**, Dept. of Patholaogy and Parasitology, Faculty of Veterinary Medicine, Chittagong Veterinary and Animal Sciences University for his trustworthy and scholastic supervision.

The author gratefully admits the help of **Prof. Dr. AMAM Zonaed Siddiki**, Dept. of Pathology and Parasitology and **Dr. Omar Faruk**, Upazilla Veterinary Surgeon, Upazilla Veterinary Hospital, Hathazari, Chittagong for their cordial supervision, scholastic guidance, and valuable suggestion during the entire period of the study.

The author expresses thanks and warmest sense of gratitude to his parents and all well-wishers.

The Author

July, 2016

Biography

I am Probir Deb, son of Mr. Prodip Kumar Deb and Mrs. Baby Deb. I passed my Secondary School Certificate examination in 2008 followed by Higher Secondary Certificate examination in 2010. Now I am an intern veterinarian under the Faculty of Veterinary Medicine in Chittagong Veterinary and Animal Sciences University. In the future, I would like to work as a veterinary practitioner and do research on Cancer in Bangladesh.