Journal of Animal Research: v.6 n.3, p. 393-399. June 2016

DOI: 10.5958/2277-940X.2016.00036.X

Kinetics of *Fasciola gigantica* Antibody Response in Naturally Infected Bovine Ensuing Levamisole and Oxyclozanide Combination Therapy

Niranjan Kumar[1], Kishan Kumar Sharma[2] and Anju Varghese[3]

[1,3]*Department of Parasitology, Vanbandhu College of Veterinary Science and Animal Husbandry, N.A.U., Navsari (Gujarat), INDIA*
[2]*Department of Microbiology; Vanbandhu College of Veterinary Science and Animal Husbandry, N.A.U., Navsari (Gujarat), INDIA*

Corresponding author: K Niranjan; Email: niruvet@gmail.com

Received: 05 January, 2015 **Accepted**: 06 May, 2016

ABSTRACT

A study was conducted to determine the kinetics of *Fasciola gigantica* infection induced antibody response against oxyclozanide-levamisole in naturally infected bovine of South Gujarat, India. Faecal analysis on before, 1st, 4th, 8th, 11th and 37th days post treatment (DPT) recorded 67.14, 34.29, 5.71, 0 and 0% parasites infected bovines. Either parasite(s) positive indigenous cattle or buffalo become parasite(s) free by 8th DPT but a lag period of 4 days had been recorded in the exotic cattle. The trematode parasites infected animals take more time to clear the infection with the used drugs, it's become 100% negative on 11th DPT while the nematodes parasites infected animals successfully clear the infection by 4th DPT and maintain it until the end of experiment. The 5 animals which showed reactivity in dipstick ELISA test following natural infection with *F. gigantica* using parasites specific diagnostic antigen, cathepsin L cysteine proteinase (CP), was subjected to indirect plate ELISA assay, the mean anti-CP antibody titer was recorded at its maximum at pretreatment in treated group positive animal when probed with CP antigen. Thereafter, a declining trend of antibody response was recorded in these naturally infected bovines which reached to undetectable level on 90th DPT in response to anthelmintic therapy while untreated animals (negative and positive animals) showed almost similar pattern of antibody response during the experiment. A significant decrease in % eosinophilia count was also observed at 37th DPT in response to treatment.

Keywords: Bovine, Kinetics, Fasciolosis, Levamisole and oxyclozanide combination

The gastro-intestinal parasites (GIP) causes significant economic losses in domestic ruminants through adverse affects on the health, weight gain, feed conversion efficiency and reproduction of animals thus severely limiting the productivity of dairy animals (Pal *et al.* 2014). Amongst various GIPs, the roundworm and trematodes are important parasites that cause economically important diseases in food producing animals (Knubben-Schweizer *et al.* 2010). Prevalence rates of GIP infection in ruminants may go up to 80-100% in different geographical region (Rahman *et al.* 2013).

Based on clinical finding alone it is not usually possible to diagnose the parasitic condition in animals in prepatent stage. Therefore, laboratory intervention will be very useful to reach on accurate early diagnosis where chemotherapeutics control of disease is required (Meaney *et al.* 2013). This approach also address recent concern is there about increasing resistance towards anti-parasitic drugs (Shalaby, 2013). Synergistic combination of the anthelmintics can be a useful method to diminish the phenomenon of drug resistance development and achieving the goal of sustainable parasites control level (Leathwick, 2012). Levamisole and oxyclozanide are administered to control nematode parasites and the adult stages of liver/ rumen fluke, respectively (Paraud *et al.* 2009). Oxyclozanide, a proton ionophore acts against the worm by inhibiting oxidative phosphorylation of cellular respiration (Lanusse *et al.* 2014). Levamisole mimics the function of acetylcholinesterase and thus causing depolarization of the ganglions and nerve cells of the worms (Yadav and Singh, 2011). Levamisole is also widely used as an immunomodulatory compound and

adjuvant in various disease conditions as it restores cell mediated immune function in peripheral T-lymphocytes and stimulates phagocytosis by monocytes (Sajid *et al.* 2006). The alkaline phosphatase is an important tegumental enzyme of trematodes and cestodes is inhibited by levamisole, defining the drug/immune system synergy phenomenon (Araujo-Montoya *et al.* 2011; Lawton *et al.* 1995). Synergistic combinations of levamisole with albendazole, mebendazole and oxyclozanide have been used to treat various parasitic infections (Bartram *et al.* 2012)

A time course analysis of *Fasciola gigantica* antibody response with respect to anthelmintic treatment of levamisole and oxyclozanide in bovine animals was conducted using parasites specific diagnostic antigen, cathepsin L cysteine proteinase (CP) in indirect enzyme linked immunosorbent assay (ELISA).

MATERIALS AND METHODS

Biological sample collection

The faecal samples, directly from rectum of studied animals, were collected in separate self sealed polythene bags in villages of Navsari district, Gujarat (India) with average monthly rainfall of 0 + 5 to 1663.77 ± 448.00 mm in winter/ summer to rainy season, respectively and the relative humidity from 29.2 ± 4.25 to 84.73± 3.44% in February to August, respectively. The blood was collected via the jugular vein into a plain test tube. Serum was separated after centrifugation and stored at -20^{0}C until further use.

Coprological examination for GIP

The faeces examined using direct smear and concentration method (sedimentation and/or floatation method) to know the presence or absence of GIP stages. Quantitative faecal examination was done to decide the egg per gram (EPG) of faeces of trematodes parasites as per modified McMaster method with certain modification (Coles *et al.* 1992).

Pre and post-anthelmintic treatment efficacy

The pre and post-anthelmintic treatment parasitic load during rainy season was carried out at animal's ware house with the use, levamisole and oxyclozanide in combination. The animals were randomly divided into treatment and control group with 70 (indigenous cattle - 46, exotic cattle - 17 and buffalo- 7) and 8 (indigenous cattle - 3, exotic cattle - 3 and buffalo - 2) animals, respectively. Each of the 70 animals of treatment group was treated with levamisole HCl and oxyclozanide with dose rate of 5 and 10 mg/kg body weight, respectively via oral route. Faecal analysis was done to judge the efficacy of anthelmintics therapy of the selected drugs at pre-treatment, 4th, 8th, 11th, 37th, 60th and 90th day post treatment (DPT).

Faecal egg count reduction test (FECRT)

The % FECR was calculated as $[(X_c - X_t)/X_c] \times 100$ where X_c and X_t is the arithmetic mean of EPG of control and treated group of animals (Coles *et al.* 1992) and [(FEC before treatment - FEC post treatment) / FEC before treatment] × 100 (Dash *et al.* 1988), respectively.

Reference sera and CP antigen for ELISA

Positive/negative reference sera and diagnostic antigen, CP for *F. gigantica* infection were procured from Helmintholgy laboratory, Division of Parasitology, IVRI, Izatnagar.

Tropical liver fluke sero-prevalence study

In dot-ELISA

The dot-ELISA test was performed as per the method described by Sriveny *et al*. (2006) with certain modifications using 200 ng of CP antigen on the rectangular piece of nitrocellulose membrane. Positive reactions were determined by the formation of a distinct brown colour dot on the paper.

In plate-ELISA

The indirect ELISA assay was performed as per the method described by Kumar *et al*. (2008) using 96 well flat bottomed polystyrene microtiter plates (Nunc, USA) coated with 2.5 µg/ml of CP antigen in carbonate-bicarbonate coating buffer. The absorbance readings were taken at 492 nm in an ELISA reader. The data

Table 1: Coprological and serological results of treated group's animals (Total = 70)

Animal details and no.		Faecal examination			dot-ELISA
		Pre-treatment	4th DPT	8th DPT	Pre-treatment
Indigenous cattle (46)	1-10	-ve	-ve	-ve	
	11	-ve	-ve	-ve	-ve
	12-13	-ve	AM (25 and 75 EPG)	-ve	
	14	-ve	AM (100 EPG), NE	-ve	-ve
	15-20	AM (4 and 2 bovines with 25 and 50 EPG, respectively)	-ve	-ve	
	21	AM (50 EPG)	-ve	-ve	-ve
	22	AM (75 EPG)	-ve	-ve	+ve (light)
	23-28	AM (1×4 and 2 bovine(s) with 25, 75, 100, 150, 250 EPG, respectively), NE	-ve	-ve	
	29-31	NE	-ve	-ve	
	32	NE	-ve	-ve	-ve
	33	NE, SC	-ve	-ve	
	34-37	AM (all bovine with 50 EPG)	AM (2 and 2 bovines with 25 and 50 EPG, respectively)	-ve	
	38	AM (50 EPG)	FA (50 EPG)	-ve	+ve (light)
	39-42	AM (1×4 bovine with 50, 75, 125 and 250 EPG)	AM (1x4 bovine with 25, 50, 100 and 150 EPG)	-ve	
	43	AM (100 EPG), FA (25 EPG)	AM (200 EPG)	-ve	+ve (severe)
	44	AM (100 EPG), FA (50 EPG), NE	AM (75 EPG)	-ve	+ve (severe)
	45-46	AM (50 and 200 EPG), NE	AM (50 EPG)	-ve	
Exotic cattle (17)	47-50	-ve	-ve	-ve	
	51	-ve	-ve	-ve	-ve
	52	-ve	AM (25 EPG)	-ve	-ve
	53	AM (25 EPG)	-ve	-ve	-ve
	54	AM (50 EPG)	-ve	-ve	-ve
	55-56	AM (50 and 100 EPG), NE	-ve	-ve	
	57	SC	-ve	-ve	
	58	AM (50 EPG)	AM (50 EPG)	-ve	
	59-60	AM (125 and 175 EPG), NE	AM (50 EPG)	-ve	
	61	AM (25 EPG), NE	AM (50 EPG)	AM (50 EPG)	-ve
Buffalo (7)	62-63	AM (50 EPG)	-ve	-ve	
	64-64	-ve	-ve	-ve	
	65	-ve	-ve	-ve	-ve
	66	-ve	AM (25 EPG)	-ve	-ve
	67	FA (50 EPG)	-ve	-ve	+ve (severe)
	68	AM (75 EPG), NE	-ve	-ve	-ve
	69	AM (25 EPG)	AM (50 EPG)	-ve	-ve
	70	AM (50 EPG)	AM (100 EPG)	AM (50 EPG)	-ve

Note: AM- Amphistomes, FA- *Fasciola gigantica*, NE- Nematodes, SC- *Schistosoma* spp. egg; -ve- negative, +ve- positive. The entire treated bovines remain uninfected with GIP on 11th, 37th, 60th and 90th DPT.

were expressed as the mean of the optical density (O.D.) recorded for duplicate samples.

Differential leucocytes count (DLC) %

The DLC % was calculated under oil immersion microscopy after staining the thin blood smear with Giemsa's stain.

Statistical analysis

Data collected was analyzed by Student's t-test or chi-square test. Values of $p < 0.05$ were accepted as significant.

RESULTS

The results of pre and post-anthelmintic therapy have been summarized in Table 1. Pre-treatment faecal analysis data showed high rate (67.14%) of prevalence of parasite(s) ($p<0.05$) in the bovines (Table 1). The faecal analysis data got inclined towards parasite(s) negative animals ($p<0.05$) on the 4th DPT and their share become more than 50% either in indigenous/ exotic cattle or riverine buffalo. The combination therapy gradually increased the share of parasites free animals up to 100% by 8th, 11th, 11th DPT in indigenous cattle, exotic cattle and buffaloes, respectively ($p<0.05$). Few animals showed the peculiarity of intermittent eggs shedding during the experiment (Table 1). The animals of untreated group remained parasites positive during the experimentation with the common feature of intermittent egg shedding. The parasites belonging to class trematode ($p<0.05$) were dominated over the parasites belonging to other helminth parasite class during the experiment (Table 1). The trematode parasites infected animals take more time to clear the infection (on 11th DPT) with the used drugs ($p<0.05$). The used drugs are highly effective against the nematodes parasites ($p<0.05$) and by 4th DPT all the infected animals successfully clear the infection (Table 1) and maintain it until the end of experiment.

Total and arithmetic mean of EPG of trematodes parasites in treated and untreated control group of animals were summarized in Table 2. At pretreatment stage for amphistomes parasites, 24, 8, 3, 2 and 2 animals have EPG in the range of 1-50, 51-100, 101-150, 151-200 and 201-250, respectively. At the start of experiment 3 animals have *F. gigantica* infection with EPG in the range of 1-50. The reduction in the EPG of the amphistomes and *F. gigantica* by 4th DPT was significant ($p<0.05$) and it become zero at 11th DPT.

Fig. 1: NCP strip depicting *F. gigantica* sero-prevalence in dot-ELISA assay

In combined drugs therapy there was 97 and 100% FECR for both amphistomes and *F. gigantica* by 8th and 11th DPT, respectively.

The observation of the reactivity of CP antigen with the sera of 20 randomly selected bovine is presented in Table 1. Antibody of tropical fasciolosis was recorded in 4 and 1 bovine(s) of treated and untreated group, respectively (Fig. 1).

Following natural infection with *F. gigantica*, all the five animals which showed reactivity in dipstick ELISA test was subjected to indirect plate-ELISA assay to monitor

Table 2: EPG of treated and untreated group's animals

EPG	Pre-treatment		4th DPT		8th DPT	
	Amphistomes	Fasciola	Amphistomes	Fasciola	Amphistomes	Fasciola
			Treated group's animal (Total = 70)			
Total	3225	125	1450	50	100	0
Arithmetic mean	46.07	1.79	20.71	0.71	1.43	0
			Untreated group's animals (Total = 8)			
Total	1000	50	475	50	450	50
Arithmetic mean	125	6.25	59.38	6.25	56.25	6.25

the kinetics of antibody response at pretreatment, 8th, 37th, 60th and 90th DPT in the environment of the selected drugs (Fig. 2). The mean anti-CP antibody titer was recorded at its max, 3.04 at the start of experiment in treated group positive animal when probed with CP antigen (Fig. 2). Thereafter, a declining trend of antibody response was recorded in these naturally infected bovines which reached to undetectable level at the end of experiment in response to anthelmintic therapy while untreated negative or positive animals showed almost similar pattern of antibody response during the experiment (Fig. 2).

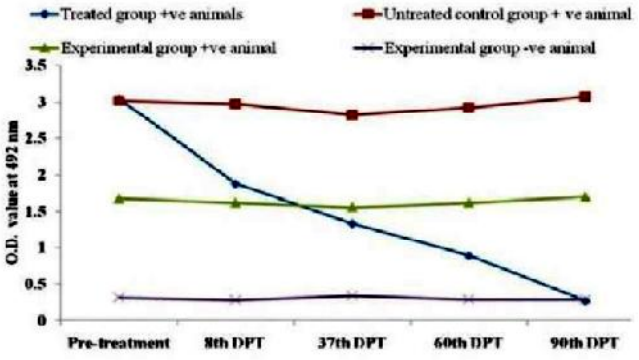

Fig. 2: *F. gigantica* antibody response in naturally infected bovine following anthelmintic therapy. Each point represents the mean O.D. 492 nm of studied animals

The mean blood cells count of 13 treated cattle for lymphocytes, monocytes, neutrophil, eosinophil and basophil was 54.62, 5.38, 16.46, 23.54 and 0% at pretreatment stage and 61.61, 7.6, 26.25, 4.54 and 0% at 37th DPT, respectively. A significant ($p<0.05$) decrease in % eosinophil count was observed at 37th DPT in response to treatment.

DISCUSSION

Seasonality of parasitic infection is well noted phenomenon, the rainy season provides most favorable condition for the rapid propagation of parasites population so the present study was undertaken during this season (Nguyen *et al.* 2012).

Our results indicated that the 2nd weeks of treatment could be very crucial in determining the effects of chemotherapeutic success of oxyclozanide and levamisole in combination. The faecal egg counts confirmed that due to long half life of the used drugs, the treatment maintain the animal, parasite free for over the 3 month monitoring period even without controlling and/or managing other factors. This finding is in accordance with Spence *et al.* (1996) who used oxyclozanide and oxfendazole in cows over the 7-month study period.

The time course analysis of antibody response in bovine against parasitic diseases can be performed by various serological assays in which indirect variant of ELISA is most widely used one. It can detect prepatent and patent parasitic infection with intermittent excretion of parasite eggs where coprological examination usually fails (Sriveny *et al.* 2006). In the present study, the antibody response in naturally infected bovines with *F. gigantica* in selected drugs environment was observed up to 90th DPT by indirect ELISA using *F. gigantica* specific, CP antigen. The dot ELISA detected the *F. gigantica* antibody in those animals who found negative in coprological examination, the field based test is widely used by various researchers to detect prepatent infection as early as 2 weeks post infection (Kumar *et al.* 2008).

Levieux *et al*. (1992) mapped chemotherapeutic success in natural bovine fascioliasis using purified specific f2 antigen of *F. hepatica* in hemagglutination test, dosed with nitroxynil and oxyclozanide 1 month later. Their f2-specific antibodies were significantly lower than those of a non-treated control group from the second month after the first treatment and continued to decline thereafter to negative values 5-6 months post-treatment.

In treated group positive animals in the present investigation quickly responded to selected chemotherapy and the titer significantly reduced to minimum on 90th DPT while the untreated positive animals maintain its antibody titer until the end of experiment. Guobadia and Fagbemi (1995) also noted significant reduction in antibody titres up to 12 weeks of post treatment in *F. gigantica* infected sheep. Reduced OD values equivalent to uninfected control group were observed only after 9 weeks in *F. gigantica* infected and oxyclozanide treated goats (Mbuh and Fagbemi, 1996). Ghosh *et al*. (2009) observed significant differences in the dynamics of antibody production in relation to 2 different *F. gigantica* specific antigens in the sera of buffaloes experimentally infected with *F. gigantica* but in naturally infected animals both the antigens showed similar pattern of antibody response following chemotherapy with

oxyclozanide. They recorded significant reduction in antibody response on 13th weeks post infection but no significant difference in the antibody titers of animals infected with 200 and 800 metacercariae was noted.

The host blood picture showing eosinophilia is an ideal indicator of active helminth infection. The degranulating mast cells release chemotactic factors which attract eosinophils cells in response to helminthic infection (Tizard, 1987). The present investigation recorded significant ($p<0.05$) reduction in eosinophil level on 37th DPT which coincide with share of parasite free animals. Pal and Dasgupta (2006) also recorded reduction in the level of eosinophil in *F. gigantica* infected buffalo in response to treatment with triclabendazole, rafoxanide and oxyclozanide with increased total erythrocytes count, hemoglobin% and packed cell volume level. Favorable changes in the blood picture in response to anthelmintic treatment were noted by many workers all round the world (Hassan *et al.* 2012; Sultana *et al.* 2015).

CONCLUSION

Overall, we propose that the synergistic effects of anthelmintics may be used to limit the rapid rate of development anti-parasitic drug resistance. Oxyclozanide chemotherapeutic success should be judge on the 2nd weeks of treatment.

ACKNOWLEDGEMENTS

The authors are thankful to the Principal Vanbandhu Veterinary College, N.A.U., Navsari for providing necessary facilities to complete the research work. We are also thankful to Dr. O.K. Raina (Principal Scientist, IVRI, Izatnagar) for providing antigen and experimental sera to conduct sero-prevalence study. A special thank is due credited to Dr. M. Prajapati for his incessant support during sample collection from the animals.

REFERENCES

Araujo-Montoya, B.O., Rofatto, H.K., Tararam, C.A., Farias, L.P., Oliveira, K.C., Verjovski-Almeida, S., Wilson, R.A. and Leite, L.C. 2011. *Schistosoma mansoni*: molecular characterization of alkaline phosphatase and expression patterns across life cycle stages. *Exp. Parasitol*., **129**(3): 284-291.

Bartram, D.J., Leathwick, D.M., Taylor, M.A., Geurden, T. and Maeder, S.J. 2012. The role of combination anthelmintic formulations in the sustainable control of sheep nematodes. *Vet. Parasitol*., **186**(3-4): 151-158.

Coles, G.C., Bauer, C., Borgsteede, F.H.M., Geerts, S., Klei, T.R., Taylor, M.A. and Waller, P.J. 1992. Methods for the detection of anthelmintic resistance in nematodes of veterinary importance. *Vet. Parasitol*., **44**(1-2): 35-44.

Dash, K.M., Hall, E. and Barger, I.A. 1988. The role of arithmetic and geometric mean worm egg counts in faecal egg count reduction tests and in monitoring strategic drenching programs in sheep. *Aus. Vet. J*., **65**(2): 66-68.

Ghosh, S., Saxena, N., Kumar, N. and Gupta, S.C. 2009. Kinetics of antibody response in experimentally infected buffaloes with *Fasciola gigantica. Indian J. Ani. Sci*., **79**(6): 537-540.

Guobadia, E.E. and Fagbemi, B.O. 1995. Time course analysis of antibody response by EITB and ELISA before and after chemotherapy in sheep infected with *Fasciola gigantica*. *Vet. Parasitol*., **58**(3): 247-253.

Hassan, M.M., Hoque, M.A., Islam, S.K.M.A., Sahaneaz Ali Khan, S.A., Hossain, M.B. and Banu, Q. 2012. Efficacy of anthelmintics against parasitic infections and their treatment effect on the production and blood indices in Black Bengal goats in Bangladesh. *Turk. J. Vet. Anim. Sci*., **36**(4): 400-408.

Knubben-Schweizer, G., Deplazes, P., Torgerson, P.R., Rapsch, C., Meli, M.L. and Braun, U. 2010. Bovine fasciolosis in Switzerland: relevance and control. *Schweiz. Arch. Tierheilkd*., **152**(5): 223-229.

Kumar, N., Ghosh, S. and Gupta, S.C. 2008. Early detection of *Fasciola gigantica* infection in buffaloes by enzyme linked immunosorbent assay and dot enzyme-linked immunosorbent assay. *Parasitol. Res*., **103**(1): 141-150.

Lanusse, C., Alvarez, L. and Lifschitz, A. 2014. Pharmacological knowledge and sustainable anthelmintic therapy in ruminants. *Vet. Parasitol*., **204**(1-2): 18-33.

Lawton, P., Sarciron, M.E. and Petavy, A.F. 1995. *Echinococcus granulosus*, *E. multilocularis* and mammalian liver-type alkaline phosphatases: a comparative study. *Comp. Biochem. Physiol. B Biochem. Mol. Biol*., **112**(2): 295-301.

Leathwick, D.M. 2012. Modelling the benefits of a new class of anthelmintic in combination. *Vet. Parasitol*., **186**(1-2): 93-100.

Levieux, D., Levieux, A., Mage, C. and Garel, J.P. 1992. Immunological detection of chemotherapeutic success in bovine fasciolosis using the specific antigen f2. *Vet. Parasitol*., **45**(1-2): 81-88.

Mbuh, J.V. and Fagbemi, B.O. 1996. Antibody and circulating antigen profiles before and after chemotherapy in goats infected with *Fasciola gigantica*. *Vet. Parasitol*., **66**(3-4): 171-179.

Meaney, M., Savage, J., Brennan, G.P., Hoey, E., Trudgett, A. and Fairweather, I. 2013. Increased susceptibility of a triclabendazole (TCBZ)-resistant isolate of *Fasciola hepatica* to TCBZ following co-incubation in vitro with the P-glycoprotein inhibitor, R(+)-verapamil. *Parasitol*., **12**: 1-17.

Nguyen, S.T., Nguyen, D.T., Van Nguyen, T., Huynh, V.V., Le, D.Q., Fukuda, Y. and Nakai, Y. 2012. Prevalence of *Fasciola* in cattle and of its intermediate host *Lymnaea* snails in central Vietnam. *Trop*. *Anim*. *Health*. *Prod*., **44**(8): 1847-1853.

Pal, P., Chatlod, L.R. and Avasthe, R.K. 2014. Epidemiology of *Haemonchus contortus* infection in goats in Sikkim. *Indian J*. *Ani*. *Sci*., **84**(8): 829-832.

Pal, S. and Dasgupta, C.K. and 2006. Haemato-biochemical profiles of buffalo in anthelmintics treatment against *Fasciola gigantica* infection. *Buffalo Bulletin*, **25**(2): 40.

Paraud, C., Gaudin, C., Pors, I. and Chartier, C. 2009. Efficacy of oxyclozanide against the rumen fluke *Calicophoron daubneyi* in experimentally infected goats. *Vet*. *J*. **180**(2): 265-267.

Rahman, H., Pal, P. and Chatlod, L.R. 2013. Incidence of gastrointestinal parasites in ruminants of organized farm in Sikkim. *Indian J*. *Ani*. *Sci*., **83**(5): 484-487.

Sajid, M.S., Iqbal, Z., Muhammad, G. and Iqbal, M.U. 2006. Immunomodulatory effect of various anti-parasitics: a review. *Parasitol*., **132**(03): 301-313.

Shalaby, H.A. 2013. Anthelmintics Resistance; How to Overcome it? *Iranian J*. *Parasitol*., **8**(1): 18-32.

Spence, S.A., Fraser, G.C. and Chang, S. 1996. Responses in milk production to the control of gastrointestinal nematode and paramphistome parasites in dairy cattle. *Aus*. *Vet*. *J*., **74**(6): 456-459.

Sriveny, D., Raina, O.K., Yadav, S.C., Chandra, D., Jayraw, A.K., Singh, M., Velusamy, R. and Singh, B.P. 2006. Cathepsin L cysteine proteinase in the diagnosis of bovine *Fasciola gigantica* infection. *Vet*. *Parasitol*., **135**(1): 25-31.

Sultana, T., Islam, M.S., Aktaruzzaman, M., Begum, F., Hossain, M.K., Lucky, N.S. and Howlader, M.M.R. 2015. Anthelmintics against ascariasis in calves inducing hematological parameters and live weight indices at sylhet dairy farm, Bangladesh. *Pharmacologia*, **6**: 386-395.

Tizard, I. 1987. Veterinary Immunology - An Introduction. 3rd end., W.B. Saunders, Philadelphia.

Yadav, P. and Singh, R. 2011. A review on anthelmintic drugs and their future scope. *Int*. *J*. *Pharm*. *Pharm*. *Sci*., **3**(3): 17-21.