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ISSN (Online): 2455-7838

SJIF Impact Factor (2016): 4.144

EPRA International Journal of

Research & Development

(IJRD)

Monthly Peer Reviewed & Indexed
International Online Journal

Volume:2, Issue:1, January 2017



Published By :
EPRA Journals

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EFFECTS OF LEVAMISOLE ON HAEMATO-BIOCHEMICAL PROFILES OF NATURALLY OCCURRING GASTROINTESTINAL NEMATODOSIS IN GAROLE SHEEP

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ABSTRACT

West Bengal, especially the 'Sunderban Delta' of South 24 Parganas district, is proud of being the home tract of a popular sheep breed, the "Garole sheep". Gastrointestinal (GI) parasitism particularly nematode infection is the major constraints of profitable sheep rearing in rural India. Parasite control has so far been predominantly relied upon the use of anthelmintic drugs. The present study was conducted to evaluate the effects of commonly used anthelmintic i.e levamisole injection against naturally occurring GI nematodosis in Garole sheep under field condition. After qualitative and quantitative screening of faecal samples of 100 sheep, thirty having faecal egg count i. e. EPG ≥ 150 were randomly selected and divided into two equal groups. Levamisole was injected @ 7.5mg kg^{-1} body weight subcutaneously to one group and the second group was kept as untreated control to harbour the naturally occurring GI nematodes. The faecal, blood and serum samples were collected aseptically once prior to treatment (0 day) and on 10th day post-treatment for estimation of haemato-biochemical parameters (Hb, PCV, TLC, serum Fe, Ca, glucose, ALP, ALT and AST) and live body weight gain or loss in both the groups was also recorded in same days. Levamisole was found to be 99.52 per cent effective against GI nematode infection. The mean values of haemoglobin (Hb) and packed cell volume (PCV), glucose level, iron (Fe), calcium (Ca) were significantly ($P < 0.05$) increased while total leucocyte count (TLC), serum alkaline phosphatase (ALP) and serum aspartate amino-transferase (AST) level were significantly decreased on 10 days post-treatment. The body weight of the Garole sheep was increased by 2.61 and 2.35 per cent, respectively, in treated and untreated control group of sheep on 10 days post-treatment. Gastrointestinal nematodes in Garole sheep can be treated successfully with a single dose of levamisole injection. The drug has direct influence on blood parameter and live weight gain of the treated animals.

KEYWORDS: Garole sheep, Gastrointestinal nematodosis, Levamisole, Haemato-biochemical changes.

INTRODUCTION

West Bengal, especially the ‘Sunderban Delta’ of South 24 Parganas district, is proud of being the home tract of a popular sheep breed, the “Garole sheep” (Sharma *et al.*, 1999). In spite of sheep being a popular livestock in this district, inadequate attention has been paid to improve its productivity. Profitable sheep rearing is posed with many constraints of which parasitic diseases; particularly gastrointestinal (GI) parasitism is the major one. GI parasitism in sheep comprised the nematode parasites as the common and major component (AINP on GIP report, 2006-07) and it is also quite prevalent in livestock of South 24 Parganas district, the breeding tract of Garole sheep. Gastrointestinal nematodosis is one of the major and widespread problems in sheep and goats affecting their health and productivity (Akerejola *et al.*, 1979). Formulation of control strategy for GI parasitism depends upon comprehensive knowledge of the epidemiological factors, which merit particular attention. Moreover, parasite control, especially the control of helminth parasites has so far been predominantly relied upon the use of anthelmintic drugs and it will continue to remain as the cornerstone of helminth control in the foreseeable future (Sanyal, 2004). Therefore, evaluation of commonly used anthelmintics against gastrointestinal nematodosis in Garole sheep with a view to select the best suited ones, is the other important area that deserves due attention.

INTRODUCTION

Agriculture is the economic backbone of West Bengal with approximately 70% people depends on it directly or indirectly for their subsistence. The livestock is an important sub-sector which contributes to solve the problems of small and marginal farmers as well as landless labourers, and plays an important role in poverty alleviation of rural people. In West Bengal, especially in coastal zone of South 24 Parganas district, is proud of being the home tract of a popular indigenous sheep breed, the “Garole sheep” (Sharma *et al.*, 1999). The sheep are primarily raised for meat and no attention is given for wool production or its improvement. Now a day, a special attention has been given on this breed due to its disease resistance power for a sustainable growth and production.

Gastrointestinal nematodosis is one of the major and widespread problems in sheep and goats affecting their health and productivity (Akerejola *et al.*, 1979). Profitable sheep rearing is there by hindered with gastrointestinal parasitism in sheep. *Haemonchus* spp., *Trichostrongylus* spp., *Oesophagostomum* spp., *Trichuris* spp. and hook worm etc. are regarded as the common and major component (Pandit *et al.*, 2009) like other ruminants. The economic losses are mainly associated with low

productivity; morbidity and occasional death (Sood, 1981). The average loss of blood in case of *Haemonchus* spp. is about 0.05 ml/adult/day with rapid fall of PCV value and decreased absorption of iron from the intestine resulting in severe anaemia and sudden death may occur from acute blood loss due to gastrointestinal nematodosis (Soulsby, 1986). The principles of control of GI parasitic diseases are mainly based on pasture and bran management and protective treatment (Roditis *et al.*, 2000). However, in this region of West Bengal, there is no organized method followed as such to control parasitic infection. A large number of broad spectrum anthelmintics are commercially available in West Bengal and levamisole is one of them. The present study was undertaken to evaluate the efficacy of this drug against gastrointestinal nematodosis alongwith some of health parameters including changes in blood picture in Garole sheep.

MATERIALS AND METHODS

Selection of study area: Sheep farm at Mohanpur in Nadia district under this university was selected as the sheep reared in this farm comprised only the selected Garole sheep and their descendants. The sheep were maintained under semi-intensive system *i.e.* in day-time they were allowed to graze in free-range field and at night-time they were kept in confinements. Routine deworming was not practiced in the sheep.

Selection and grouping of the animals: A total of 150 Garole sheep, above four-months of age, were coprologically screened for the presence of gastrointestinal nematode eggs by qualitative faecal examination using standard salt floatation technique (Soulsby, 1982) on two occasions at an interval of three days. The sheep found positive for gastrointestinal nematode eggs were then subjected to quantitative faecal examination by modified McMaster’s technique (Soulsby, 1982). The sheep having EPG \geq 150, were identified and thirty such sheep were selected for anthelmintic evaluation. Selected sheep were randomly divided into two equal groups (n = 15) *i. e.* Group I and II, for evaluating the efficacy of a single dose of levamisole.

Anthelmintic treatment: Out of the two groups, sheep of first groups were treated with levamisole (Lemasol-75®) @ 7.5 mg kg⁻¹ body wt. subcutaneously and second group served as infected untreated control throughout the study period.

Collection and examination of faecal samples: Freshly voided faecal pellets or per-rectal faecal samples were collected in 30 ml wide-mouthed plastic sample bottles, brought to the laboratory and preserved by adding sufficient amount of 10 % formalin till further examination. Pre-treatment samples were collected twice at three days

intervals and the post-treatment samples were collected 10 days after the treatment as recommended by Coles *et al.* (1992). The faecal egg count (FEC), *i.e.* EPG in respect of nematodes (*Strongyle*, *Strongyloides* spp., *Trichuris* spp.) were determined quantitatively by modified McMaster's Technique (Soulsby, 1982).

Anthelmintic efficacy: The efficacy of anthelmintics under field condition was studied following the method described by Coles *et al.* (1992).

Collection and examination of blood and serum samples: For estimating the haematological

parameters *i.e.* haemoglobin (Hb), packed cell volume (PCV), total erythrocyte count (TEC), total leucocyte count (TLC), and serum biochemical parameters *i.e.* total serum protein (TSP), serum albumin (SA), serum globulin (SG), serum albumin and globulin ratio (SA: SG), serum alkaline phosphatase (AP), serum alanine amino-transferase (ALT), serum aspartate amino-transferase (AST), serum glucose level, iron (Fe), calcium (Ca) and inorganic phosphorus (P) changes due to anthelmintic treatment against naturally occurring gastrointestinal nematodosis, blood and serum samples were collected once prior and after 10 days of anthelmintic treatment from each group. All the parameters were estimated by standard method/protocol.

Recording of body weight: The body weight changes in the treated groups as compared to the infected untreated control group were also taken into account for assessment of anthelmintic efficacy. The individual body weight of all the sheep was measured with the help of floor balance at the time of collection of faecal samples *i.e.* once prior to the anthelmintic treatment and once on 10th day post-treatment.

Statistical analysis: All the parameters for each group were compared (Analyze - Compare Means) for the mean value along with standard error (S.E). Then they were analyzed separately (*i.e.* between groups and between post-infection period) by Duncan method (One way ANOVA) and the significance (P value) was recorded at 5% level and 1% level.

The complete statistical analysis was done with the help of SPSS Windows Version 10.0.

RESULTS AND DISCUSSION

Anthelmintic treatment has been serving as the most effective mean for the control and amelioration of helminth infections in livestock including sheep. But the prolonged and indiscriminate use of anthelmintics has led to the emergence of anthelmintic resistance, which is a major constraint for nematode control throughout the world including India (Singh *et al.* 2002). However, this problem has not been recorded so far in West Bengal.

It is evident from Table-1 that the mean faecal egg counts (FEC) in the treated groups were significantly ($P < 0.01$) reduced compared to the respective pre-treatment FEC, whereas the difference of pre and post treatment FEC in the control group (Gr-II) was non-significant. The percent efficacy of the levamisole was 99.52 per cent, against gastrointestinal nematodes in Garole sheep. Although the efficacy of the anthelmintic, as judged by post-treatment FEC, was not significantly different within the groups, the post-treatment FEC in untreated control group (Gr-II) was significantly ($P < 0.01$) higher as compared to the treated group (Gr.I). Earlier evaluations of levamisole against gastrointestinal nematodosis in sheep under unorganized and semi-intensive farming system revealed that they were effective beyond the desired level (Panda *et al.*, 2003; Gomathinayagam *et al.*, 2004; Garg *et al.*, 2007).

Table-1: The mean (\pm SE) faecal egg count (EPG), haemato-biochemical and body weight changes in Levamisole treated and untreated control groups of Garole sheep

Parameter studied	Pre-treatment value (on day 0)		Post-treatment value (on 10 th DPT)	
	Gr-I	Gr -II	Gr-I	Gr-II
EPG	663.33 \pm 83.16	526.67 \pm 67.76	3.33 \pm 3.33	696.67 \pm 68.74**
Hb (g%)	8.62 \pm 0.73	9.10 \pm 1.38	9.16 \pm 0.37	8.70 \pm 0.36*
PCV (%)	26.46 \pm 0.84	27.24 \pm 0.48	28.22 \pm 0.57	26.30 \pm 1.92*
TEC (X 10 ⁶ /cu mm)	11.91 \pm 0.05	12.15 \pm 0.25	12.24 \pm 0.08	11.46 \pm 0.22**
TLC (X 10 ³ /cu mm)	12.15 \pm 0.48	12.02 \pm 0.23	11.70 \pm 0.21	12.38 \pm 0.15
TSP (gm/dl)	6.97 \pm 0.12	7.43 \pm 0.39	7.42 \pm 0.31	6.96 \pm 0.15*
SA (gm/dl)	3.78 \pm 0.22	3.43 \pm 0.15	3.87 \pm 0.20	3.26 \pm 0.16
SG (gm/dl)	3.19 \pm 0.27	3.99 \pm 0.28	3.55 \pm 0.20	3.70 \pm 0.52
SA:SG	1.25 \pm 0.25	0.87 \pm 0.05	1.10 \pm 0.07	0.91 \pm 0.09
ALP (IU/L)	72.38 \pm 3.12	76.25 \pm 3.01	70.98 \pm 2.51	88.44 \pm 3.91**
ALT (IU/L)	27.78 \pm 0.98	28.17 \pm 1.06	26.57 \pm 1.07	31.40 \pm 0.82**
AST (IU/L)	66.95 \pm 2.66	89.20 \pm 4.73	66.86 \pm 2.17	103.16 \pm 7.37**
Glucose (g%)	65.94 \pm 2.28	63.37 \pm 2.93	70.66 \pm 1.83	61.13 \pm 2.38**
Fe (μ g/dl)	127.38 \pm 4.74	127.06 \pm 3.20	137.9 \pm 7.69	117.26 \pm 9.09*
Ca (mg/dl)	5.85 \pm 0.32	6.08 \pm 0.32	6.16 \pm 0.29	5.74 \pm 0.30*
P (mg/dl)	3.50 \pm 0.20	4.27 \pm 0.32	3.82 \pm 0.22	3.94 \pm 0.28
Body weight changes (in Kg)	9.20 \pm 0.64	8.94 \pm 0.43	9.64 \pm 0.65	9.15 \pm 0.42

Gr-I = Levamisole treated group of sheep

** = Significant at 1% level ($p < 0.01$),

DPT = Day post-treatment

Gr-II = Untreated control group of sheep

* = Significant at 5% level ($p < 0.05$)

SE = Standard error

Anthelmintic treatment and heamatological changes:

The mean Hb concentration was significantly ($P < 0.05$) increased on 10th day after treatment with all the three anthelmintics compared to the infected

untreated control group. However, the Hb concentration in the three treated groups did not differ significantly ($P > 0.05$). A similar pattern of changes in PCV and TEC values due to treatment was also observed (Table- 2).

However, the TLC values on 10 DPT with all the three anthelmintics were significantly ($P < 0.05$) lower than the untreated control group, although the difference between any two treated groups was not significant. All the three anthelmintics improved the Hb, PCV and TEC values but TLC values had a declined tendency after 10 day post-treatment. This finding commensurate the earlier findings of Rajguru *et al.* (2002), and Arora *et al.* (2003).

Anthelmintic treatment and serum protein level:

The treatment of gastrointestinal nematode infected sheep with ivermectin, levamisole and albendazole significantly ($P < 0.05$) improved the TSP levels compared to the untreated sheep (Table-3), although the difference within the treated groups was not significant. The SA and SG levels and SA: SG values did not change significantly due to treatment with all the three anthelmintics. There was significant amelioration of the TSP level due to anthelmintic treatment without significant effect on SA, SG and SA: SG values. Similar findings have been reported earlier by Badrie and Kamenov (1982), Tanwar and Mishra (2001).

Anthelmintic treatment and enzyme activity:

The activities of AP, ALT and AST in the three treated groups were significantly ($P < 0.01$) reduced compared to untreated control sheep after 10 days following the treatment (Badrie *et al.*, 1985 and Chakraborti and Lodh, 1994).

Anthelmintic treatment and serum glucose level:

Serum glucose level recorded a significant ($P < 0.01$) increase due to treatment of the gastrointestinal nematode infected sheep with ivermectin, levamisole and albendazole compared to untreated sheep but there was no significant difference within the three treated groups. The values in the treated groups increased significantly ($P < 0.05$), compared to the untreated control group. Interestingly, the glucose level in untreated control group was significantly reduced on 10th day (61.13) compare to the initial value (63.37). Decreased level of serum glucose in lambs infected with *O. venulosum* has been reported earlier by Badrie *et al.*, (1985). In the ivermectin treated group the glucose level significantly ($P < 0.01$) increased on 10 DPT (74.53) compared to the pre-treatment level (67.40).

Anthelmintic treatment and serum mineral concentration:

Treatment with albendazole significantly ($P < 0.05$) increased serum Fe level, although the effect of treatment with ivermectin and levamisole was non-significant. In case of Ca concentration it was evident that treatment with ivermectin and albendazole had significant ($P < 0.05$) positive effect but the effect of

levamisole treatment was non-significant ($P > 0.05$). The difference in serum Ca values was non-significant within the three treated groups. Interestingly, the serum inorganic phosphorous (P) level did not change significantly due to treatment with all the three anthelmintics. Decreased level of Ca and P due to *T. colubriformis* (Brown *et al.*, 1989), of Fe and Ca due to haemonchosis (Brar *et al.*, 1998; Sangwan and Sangwan, 2000) have also been reported earlier in infected animals compared to the treated animals.

Anthelmintic treatment and body weight changes:

The treatment of gastrointestinal nematode infected sheep with all the three anthelmintics resulted in a gain in the body weight, although this gain was not statistically significant compared to the untreated control. The reduced body weight in infected sheep indicates negative impact of gastrointestinal nematodosis on the growth rate as noted by Hayat *et al.* (1996) and Uriarte *et al.* (2003).

The sheep used in the present study for evaluating the anthelmintics did not receive regular anthelmintic treatments and all the three anthelmintics evaluated, had the desired efficacy. Additionally, there was improvement in the haemato-biochemical parameters and body weight in the treated groups.

Acknowledgement

Authors are thankful to the Dean, Faculty of Veterinary and Animal Sciences, West Bengal University of Animal and Fishery Sciences, for providing necessary research facilities for the present study. The financial assistance provided by the I.C.A.R. in the form of a research project entitled "A.I.N.P. on gastrointestinal parasitism" under which this work was conducted, is also gratefully acknowledged.

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