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Dynamic Dispersal of Haemonchosis, its Treatment and Effect on Blood Profile of Small Ruminants of Lodhran District, Punjab, Pakistan

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ABSTRACT

This study was designed to determine dynamic dispersal, treatment of *Haemonchus contortus* and its effect on blood profile of small ruminants in district Lodhran, Punjab. Out of total 646 animals, 30% (97/323) sheep and 25% (81/323) goats were found positive. The prevalence was found associated with seasons and body condition score. They were treated with the combination of triclabendazole and levamisole, *Mallotus philippensis* and *Fumaria indica* Triclabendazole and levamisole combination was the most effective treatment in order followed by kamila (*Mallotus philippensis*) and shahtrah (*Fumaria indica*).The statistical analysis of blood profile showed significant (P<0.05) decrease in red blood cells, haemoglobin, pack cell volume, total serum protein concentration, serum albumin and significant (P<0.05) increase in eosinophil number and serum enzymes level in infected animals. In conclusion, *H. contortus* may be considered as endemic in study area.

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Authors' Contributions

HMQ designed the study, collected and analyzed the samples. MA and AZD analyzed the data. AHS wrote the article.

Key words

***H. contortus*, Sheep, Goat, Prevalence, Treatment, EPG, Blood profile.**

INTRODUCTION

Gastro-intestinal nematodes are common in small ruminants of developing countries like Pakistan (Strain and Stear, 2001; Saddiqi *et al.*, 2010). Among them, *Haemonchus contortus* is a voracious blood sucking nematode and responsible for huge economic losses (Raza *et al.*, 2007; Saddiqi *et al.*, 2010) through anemia, diarrhea, weight loss and death of animals (Ejlertsen *et al.*, 2006; Squires *et al.*, 2011; Nabi *et al.*, 2014). Each worm of *H. contortus* sucks about 0.05 ml of blood per day from stomach (Raza *et al.*, 2009; Tasawar *et al.*, 2010; Qamar and Maqbool, 2012). It has also effects on digestive efficiency which can lead to loss of meat (27%) and wool (40%) in sheep and goats (Iqbal and Jabbar, 2005; Bachaya *et al.*, 2006; Mushtaq *et al.*, 2011). The prevalence range of *H. contortus* is in between 24.6% to 80.64% in Pakistan (Al-Shaibani *et al.*, 2008; Asif *et al.*, 2008). *H. contortus* infection has also been reported to cause hematological and biochemical abnormalities (Asif *et al.*, 2008; Ijaz *et al.*, 2009; Hassan *et al.*, 2013). Clinical signs and presence of eggs in feces are the two major tools for the diagnosis of haemonchosis. The treatment of haemonchosis is based upon anthelmintic drugs (McKellar and Jackson, 2004), but the worms seem to have developed resistance against these drugs (Mortensen *et al.*, 2003; Kaplan, 2004). So, herbal medicine may be considered as one of the effective treatment along with anthelmintic. The anthelmintic activity of various plants has been studied by different researchers (Githiori *et al.*, 2004; Marie-Magdeleine *et al.*, 2010; Kamaraj *et al.*, 2011). The present study was designed to check the prevalence, intensity, treatment and effect of haemonchosis on blood profile of sheep and goats in district Lodhran, Punjab, Pakistan.

MATERIALS AND METHODS

Prevalence study was conducted from April 2013 to March 2014 in sheep and goats reared by small holder livestock farmers in Lodhran. Different animal farms were visited on weekly and monthly basis during which 646 small ruminants (n=323 sheep and n=323 goats) were randomly selected and sampled. About 5 grams of fecal sample was collected directly from the rectum of each sheep and goat after wearing disposable gloves. The samples were stored in 10% formalin, labeled and dispatched to Medicine Laboratory, University of Veterinary and Animal Sciences, Lahore. The fecal samples were examined through direct smear method while EPG was performed using modified McMaster Technique. The identification of *H. contortus* egg was based on morphology (Soulsby 2005; Kuchai *et al.*, 2012).

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A total of 60 animals (n=30 sheep and n=30 goats) were randomly selected out of which 48 (n=24 sheep and n=24 goats) were naturally infected with *H. contortus* and 12 (n=6 sheep and n=6 goats) were negative for *H. contortus*. Animals were randomly divided into five groups each of 6. For chemotherapy, the animals in group A was treated with triclabendazole plus levamisole (Trimax®, Prix Pharmaceutica (Pvt) Ltd) at 12 mg +7.5 mg/kg orally, while the members in group B were given kameela (*Mallotus philippensis*) at 24mg/kg orally. The sheep and goats in group C were given shahtra (*Fumaria indica*) at 35 mg/kg orally. The members in group D served as control positive and the members in group E served as control negative. Fecal samples were collected at day 0 (pre-medication) and then at day 3, 7, 14, and 21 (post-medication) for egg per gram (EPG). EPG was counted by McMaster technique as detailed by Soulsby (2005). The efficacy of the drugs was calculated on the basis of reduction in EPG measured as per formula (Varady *et al.*, 2004).

$$\text{Drug efficacy} = \frac{\text{Pre Treatment EPG} - \text{Post Treatment EPG}}{\text{Pre Treatmnt EPG}} \times 100$$

Blood Profile

For this purpose 10 ml blood sample was collected from each member of groups A, B, C, D and E at day 0 (before treatment) and day 21 (after treatment). After collection blood was stored in test tubes having tubes containing anticoagulant for biochemical analysis and without anticoagulant for haematological analysis using haematological and biochemistry analyzers at University Diagnostic Laboratory.

Statistical analysis

The data of prevalence was analyzed by frequency analysis using chi-square test. The percentages and 95% confidence limit for some parameters were determined and where appropriate odd ratio was also computed. Data on chemotherapy and hemogram was analyzed by one way Analysis of Variance (ANOVA) using Statistical Package for Social Sciences (SPSS) version 17.0. A probability level ≤ 0.05 was considered as statistically significantly different.

RESULTS AND DISCUSSION

Dynamic dispersal

Table I shows overall prevalence, sex wise, breed wise, feeding system wise and season wise prevalence of *H. contortus* in sheep and goats of District Loadhran. *H. contortus* is of high economic significance due to its high prevalence and blood sucking habit (Aumont *et al.*, 1997; Notter *et al.*, 2003) especially under warm and wet conditions. The findings of our study are congruent with the results reported by Muzarab *et al.* (1980), Maqsood *et al.* (1996) and Tariq *et al.* (2003, 2008) were 36.8%, 47.1%, 38% and 59.6% in small ruminants, respectively. These minor differences in the range of prevalence may be due to environmental and managerial conditions. Nearly, similar trend of sex wise prevalence of *H. contortus* was recorded in males (84.6%) as compared to females (72.1%) in sheep. These significantly differences were due to lack of resistance of animals to *H. contortus* infection and environmental factors may contribute to this (Tasawar *et al.*, 2010). Similarly the higher prevalence was illustrious in summer, followed by autumn, spring and winter by Nginyi *et al.* (2001) in Kenya, Shahadat *et al.* (2003) in Bangladesh, Khajuria and Kapoor (2003) in India and Lateef *et al.* (2005) in Pakistan. There are several aspects that contribute the disease commencement like humid, warm and wet grazing season, the more time animals used on pasture, poor husbandry practices, ineffective choice of de-wormers and increased anthelmintic resistance.

Intensity of H. contortus

Table II shows EPG values of sheep and goats in different groups at at day 0 (pre-treatment) 3, 7, 14 and 21 (post treatment).

Chemicals are mostly used for the treatment and control of gastrointestinal parasites (Ancheta *et al.*, 2004; McKellar and Jackson, 2004; Ghisi *et al.*, 2007). *Mallotus philippinensis* usually known as kamila in traditional medicine is anthelmintic and it has also effects against tape worm infections, parasitic skin infections, eye problems (Sharma and Varma, 2011; Ahmed and Siddiqa, 2013). *Fumaria indica* frequently known as Shahtrah, it has many medicinal properties in folk medicine as an anthelmintic, diuretic, laxative and stomachic activities and it is also used for purification of blood in traditional medicine system (Gupta *et al.*, 2012).

Efficacy of drugs against H. contortus

Table II also shows that combination of triclabendazole and levamisole was most effective treatment for *H. contortus* followed by kamila (*Mallotus philippensis*) and shahtrah (*Fumaria indica*) both in sheep and goats.

Hematological values of sheep and goats

Hematological parameters in different groups of sheep and goats are given in Table III. At day 21 RBC's count, hemoglobin, PCV, MCV, MCH, MCHC, platelets and lymphocyte count increased significantly ($P<0.05$) in groups A, B and C of sheep and goats compared to group D whereas WBC count, monocyte count and granulocytes

Table I.- Prevalence and egg per gram count of *H. contortus* in sheep and goats in Lodhran.

Species/sex/ age/breed	No. of animal	Positive (%)	95% CI	Odd Ratio/ reciprocal	MH Chi-Sq P-value
Sheep	323	97 (30.03)	25.22-35.20	1.28/0.78	-
Goats	323	81 (25.07)	20.58-30.02		
Sheep: Sex					
Male	110	25 (23.00)	15.62-31.25	0.58/1.74	-
Female	213	72 (33.80)	27.69-40.36		
Breed					
Cholistani	110	36 (32.72)	24.45-41.90		
Thali	101	27 (26.73)	18.79-35.99	-	P = 0.352
Buchi	112	34 (30.36)	22.38-39.34		
Feeding system					
Stall feeding	63	16 (25.40)	15.82-37.19		
Grazing	180	59 (32.77)	26.22-39.89	-	P = 0.435
Stall+Grazing	80	22 (27.50)	18.57-38.03		
Seasons					
Summer	110	57 (51.82)	42.49-61.05		
Spring	95	23 (24.21)	16.40-33.57	-	P = 0.000
Autumn	53	10 (18.86)	10.00-31.06		
Winter	65	7 (10.77)	4.83-20.14		
Goats: Sex					
Male	90	19 21.11)	13.61-30.44	0.93/1.07	-
Female	233	52 (22.32)	17.32-28.00		
Breeds					
Nachi	92	22 (23.91)	16.03-33.41		
Beetal	125	32 (25.60)	18.53-33.79	-	P = 0.404
Teddy	106	27 (25.47)	17.87-34.40		
Feeding system					
Stall feeding	55	11 (20.00)	10.99-32.10		
Grazing	195	54 (27.69)	21.75-34.29	-	P = 0.460
Stall+Grazing	73	16 (21.92)	13.55-32.48		
Season					
Summer	125	54 (43.20)	34.72 -51.99		
Spring	96	18 (18.75)	11.87-27.49	-	P = 0.000
Autumn	40	4 (10.00)	3.26-22.38		
Winter	62	5 (8.06)	3.01-16.97		

Summer (May-Aug); Spring (Feb-April); Autumn (Sep-Oct); Winter (Nov-Jan)

Table II.- EPG values of *H. contortus* at various days and in sheep and goats.

Groups	Animal	EPG values at various days after treatment				
		0day	**3rd day**	**7th day**	**14th day**	**21st day**
A (Triclabendazole + Levamisole)	Sheep	1867 ± 176.38^{a}	1433 ± 130.81^{a}	817 ± 65.41^{a}	633 ± 61.46^{a}	167 ± 61.46^{a}
	Goat	1717 ± 60.09^{a}	1333 ± 33.33^{a}	967 ± 55.78^{a}	650 ± 42.817^{a}	233 ± 55.78^{a}
B (Mallotus philippensis)	Sheep	1800 ± 73.03^{ab}	1533 ± 80.28^{ab}	1117 ± 87.24^{b}	750 ± 76.38^{ab}	367 ± 55.78^{ab}
	Goat	1783 ± 47.72^{ab}	1500 ± 51.63^{ab}	1200 ± 63.24^{ab}	800 ± 63.24^{ab}	417 ± 47.72^{ab}
C (*Fumaria indica*)	Sheep	1750 ± 76.38^{abc}	1500 ± 73.03^{abc}	1200 ± 57.74^{c}	917 ± 47.73^{c}	533 ± 55.78^{bc}
	Goat	1733 ± 55.78^{abc}	1567 ± 61.46^{bc}	1300 ± 73.02^{bc}	983 ± 47.73^{bc}	600 ± 51.64^{bc}
D (Positive control)	Sheep	1733 ± 88.19^{abcd}	$1783.\pm70.32^{bcd}$	1833 ± 80.28^{d}	1917 ± 79.23^{d}	2017 ± 70.32^{d}
	Goat	1767 ± 71.49^{abcd}	1783±70.32d	1817 ± 70.31^{d}	1883 ± 70.32^{d}	1983 ± 70.32^{d}
E (Negative control)	Sheep	0.00 ± 0.00^{e}	0.00 ± 0.00^{e}	0.00 ± 0.00^{e}	0.00 ± 0.00^{e}	0.00 ± 0.00^{ae}
	Goat	0.00 ± 0.00^{e}	0.00±0.00e	0.00 ± 0.00^{e}	0.00 ± 0.00^{e}	0.00 ± 0.00^{e}

Values in same column having different superscript letters are statistically significantly (P<0.05) different.

Table III.- Hematological values in different groups at day 0 and 21 in sheep and goats (Mean±SD).

Groups	Days	WBCs ($\times10^9$/l)	RBCs ($\times10^{12}$/l)	Hb (g/dl)	PCV (%)	MCV (fl)	MCH (pg)	MCHCz(g/dl)	PLT1 ($\times10^9$/l)	Lymph (%)	Mono (%)	Granul (%)
Sheep												
A (n=6)	0	41.05±10.21*	7.53±1.01*	6.98±1.34*	20.58±2.60*	24.16±2.22*	6.56±0.52*	23.61±2.65*	*183.00±69.45	78.23±8.31*	6.43±1.98*	70.66±8.47*
	21	7.83±1.50*	11.94±1.84*	12.73±1.48*	42.5±9.28*	33.55±3.12*	10.00±0.97*	32.30±0.88*	*544.00±149.04	55.95±9.82*	3.70±0.86*	33.08±6.60*
B (n=6)	0	39.43±10.38*	6.50±1.142*	6.08±0.67*	19.75±1.58*	22.33±1.15*	5.58±1.01*	21.98±1.43*	*192.50±70.51	79.33±10.34*	4.11±1.18*	72.16±9.17*
	21	8.65±1.88*	12.11±1.44*	11.83±1.89*	40.83±7.86*	33.68±2.95*	10.68±0.97*	32.58±0.52*	*558.67±64.05	55.13±9.11*	3.01±1.27*	28.56±9.60*
C (n=6)	0	37.60±8.96*	5.19±.86*	5.16±0.68*	18.91±2.54*	21.56±0.85*	4.91±0.67*	18.45±1.45*	*165.33±45.74	80.16±5.58*	4.38±1.01*	72.00±10.39*
	21	6.98±1.38*	11.95±1.72*	11.83±1.54*	50.57±11.17*	34.08±3.42*	9.93±0.79*	32.36±1.12*	*556.50±101.08	51.26±4.53*	2.51±0.52*	29.40±7.84*
D (n=6)	0	41.26±13.57**	5.06±0.75**	5.01±0.85**	14.53±2.51**	18.76±1.88**	4.78±0.78**	19.96±2.35**	**152.83±27.13	65.95±11.64**	2.26±0.77**	70.01±8.03**
	21	44.26±8.14**	4.65±0.56**	4.73±0.48**	13.01±1.97**	17.26±1.41**	4.61±0.57**	19.63±2.79**	**145.50±24.77	66.78±12.86**	2.26±0.77**	77.18±8.89**
E (n=6)	0	8.05±1.54**	12.65±1.57**	12.96±1.22**	49.53±11.55**	33.63±2.01**	10.18±1.1**	32.61±0.79**	**437.83±153.62	59.28±8.51**	4.01±1.32**	35.90±8.18**
	21	8.05±1.54**	12.65±1.57**	12.96±1.22**	49.53±11.55**	33.63±2.01**	10.18±1.1**	32.61±0.79**	**437.83±153.62	59.28±8.51**	4.01±1.32**	39.21±5.27**
Goats												
A (n=6)	0	34.45±8.17*	6.34±0.99*	6.40±0.35*	23.16±1.16*	20.46±1.82*	5.00±0.43*	27.38±2.00	*247.00±26.43	35.00±3.02*	5.00±0.44*	70.66±8.47*
	21	9.13±0.86*	13.40±1.54*	11.68±1.16*	33.83±4.07*	33.08±3.04*	10.15±1.03*	32.21±1.00	*403.33±37.10	50.03±1.00*	3.16±0.42*	30.08±6.00*
B (n=6)	0	35.28±6.03*	6.10±1.10*	6.13±0.21*	23.33±1.21*	19.00±1.49*	5.00±0.43*	27.00 ±3.00	*230.00±26.39	34.00±4.15*	4.38±1.00*	72.16±9.17*
	21	10.21±0.94*	13.40±0.90*	11.85±1.39*	37.00±6.16*	34.16±4.07*	10.28±1.37*	32.26±1.01	*383.33±19.91	51.00±8.00*	3.00±1.01*	28.00±9.30*
C (n=6)	0	27.50±5.74*	5.01±0.81*	6.15±0.18*	23.33±1.21*	26.00±1.21*	5.00±0.24*	29.00 ±2.00	*223.50±22.81	34.40±4.00*	4.11±1.00*	72.00±10.39*
	21	9.86±1.13*	13.53±0.55*	11.35±1.58*	38.16±5.63*	38.16±5.00*	10.23±1.03*	32.28±1.00	*394.16±28.35	59.00±7.00*	2.00±1.08**	26.40±6.84*
D (n=6)	0	37.00±6.69**	5.30±0.75**	6.10±0.12**	23.00±0.89**	27.00±1.19**	5.00±0.14**	37.00 ±4.00	**212.16±21.70	37.00±4.00**	2.00±1.08**	70.01±8.03**
	21	43.50±7.23**	5.06±0.74**	5.73±0.25**	20.33±1.50**	28.00±1.19**	5.00±0.14**	37.00 ±4.00	**202.16±21.70	37.00±4.00**	4.00±1.00**	77.18±8.89**
E (n=6)	0	8.33±0.60**	11.48±1.56**	9.43±0.58**	38.66±7.96**	33.00±2.14**	6.04±0.35**	32.13±1.00	**442.50±30.31	49.33±6.43**	4.00±1.00**	36.90±8.19**
	21	8.33±0.60**	11.48±1.56**	9.43±0.58**	38.66±7.96**	33.00±2.14	6.04±0.35**	32.13±1.00	**442.50±30.31	49.33±6.43**	3.70±0.62	36.90±8.19**

Values having different superscripts letters in each column are statistically significantly different (P<0.05) *indicates that values are statistically significantly different (P<0.05) **indicate that values are statistically non significantly different (P>0.05)

Table IV.- Serum biochemistry values in different groups at day 0 and 21 in sheep and goat (Mean±SD).

Group	Days	Total serum protein (g/dl)	Albumin (g/dl)	ALT (u/l)	AST (u/l)
Sheep					
A (n=6)	0	2.85±0.76*	1.83±0.28*	55.33±5.68*	137.00±8.60*
	21	6.83±0.38*	3.05±0.24*	28.66±5.68*	96.83±18.91*
B (n=6)	0	2.78±0.64*	1.78±0.27*	56.66±7.60*	152.16±8.23*
	21	6.98±0.49*	3.13±0.39*	32.66±5.53*	85.50±11.32*
C (n=6)	*0*	2.95±0.73*	1.88±0.14*	58.33±6.97*	151.83±13.01*
	21	6.00±0.24*	2.65±0.25*	37.83±5.30*	108.83±10.22*
D (n=6)	0	2.55±0.52**	1.36±0.41**	61.33±7.76**	157.83±19.81**
	21	2.61±0.62**	1.48±0.47**	64.33±11.32**	172.66±25.37**
E (n=6)	0	6.86±0.54**	3.40±0.14**	29.50±5.92**	82.66±20.37**
	21	6.86±0.54**	3.40±0.14**	29.50±5.92**	2.66±20.37**
Goats					
A (n=6)	0	3.35±0.62*	1.96±0.24*	70.83±6.76*	248.83±6.30*
	21	6.93±0.33*	3.11±0.25*	34.16±6.43*	137.66±34.58*
B (n=6)	0	2.75±0.45*	1.61±0.34*	62.16±6.30*	255.16±10.77*
	21	7.00±0.34*	3.56±0.57*	36.50±7.68*	194.83±11.33*
C (n=6)	0	3.65±0.81*	1.46±0.37*	71.83±6.73*	151.83±13.01*
	21	6.56±0.38*	3.63±0.78*	37.83±5.30*	209.00±9.87*
D (n=6)	0	2.55±0.52**	2.01±0.48**	72.00±9.01**	267.66±11.48**
	21	2.46±0.64**	1.35±0.47**	65.00±10.27**	172.66±25.37**
E (n=6)	0	6.81±0.26**	3.45±0.36**	41.83±9.02**	156.16±17.22**

n= No. of animals; Values having different superscripts letters in each column are statistically significantly different (P<0.05) *indicates that values are statistically significantly different (P<0.05); **indicate that values are statistically non significantly different (P>0.05)

count were significantly decreased (P<0.05) in sheep and goats of groups A, B and C compared to group D. Major effects of parasite on indigenous animals are severe anemia along with hematological disturbances (Iqbal *et al.*, 1998). After treatment with different drugs the quality of blood loss by the parasites was minimized as reported by Yacob *et al.* (2008). The value of circulating eosinophil count was higher in infected animals. The relationship of reduced packed cell volume and eosinophilia was observed with each other (Woolaston *et al.*, 1996).

Serum biochemical values of sheep and goats

The serum biochemical values in different groups of sheep and goats are given in Table IV. At day 21 albumin and total serum protein were significantly increased (P<0.05) in groups A, B and C of sheep and goats compared to D whereas ALT and AST was significantly reduced (P<0.05) in goats of groups A, B and C of sheep and compared to D.

H. contortus has serious effect on serum biochemistry and enzymatic assays like ALP, AST and ALT. It has been reported higher significantly in small ruminants (Hassan *et al.*, 2013). There is a change in total serum protein (TSP) level, synthesis of proteins in liver was found to be increased and was suggested to be due to the loss of plasma protein into the intestine as a result of increased mucosal permeability caused by *H. contortus* and many more parasites (Bahrami *et al.*, 2011). Based on these results, it may be concluded that small ruminants of the selected study area are under the high risk of the *H. contortus*, which may be minimized by controlling the various associated determinants and applying the suitable treatment in combination.

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