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Study on Hemato-biochemical Alterations, Oxidative Stress and Epidemiology of Parasitic Infestation along with Therapeutic Effect of Eprinomectin in Sheep in South Punjab

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ABSTRACT

Livestock plays a pivotal role in the rural economy because almost 35-40 million population in the villages are getting 30-40% of their income from livestock resources. The main health concern of sheep is parasitic infestation, which causes a decrease in weight gain, poor body condition, and decrease in birth weights. This study has been designed to explore hematobiochemical alterations, oxidative stress and epidemiology of parasitic infestation along with therapeutic effect of eprinomectin in sheep. A total of 320 sheep of both sexes were selected to collect fecal and skin-scraping samples separately. For therapeutic trials, healthy sheep (n=6) and infected sheep with parasites (n= 6) were selected to apply eprinomectin tropically. The blood samples of sheep were collected from both groups on 1st and 14th days for complete blood count and serum biochemistry. The results showed that overall prevalence of ectoparasites was 30% in sheep. There was no significant difference in area, age and sex wise prevalence for ectoparasites but tick infestation (42.8%) was significantly (P<0.05) higher than lice (17.8%) and mites (23.5%). Overall prevalence of endoparasites in sheep was 42.5%. There was no significant difference in area and species-wise, but female (47.4%) was significantly more infected than male (35.4%), and old animals (68.88%) were significantly (P<0.05) more infected than suckler (20%) and adult (36.47%) animals. On 1st day, WBC, RBC, Hb, HCT and MCV were significantly (P<0.05) different between healthy and infected groups. While, after the administration of the eprinomectin on 14th day WBC and Hb were significantly (P<0.05) different and other parameters were same between healthy and treatment groups. After the administration of eprinomectin, total protein, albumin, glutamic-pyruvic transaminase, and serum glutamic-oxaloacetic transaminase were same (P>0.05) between healthy and treatment groups on 14th day. These results indicated that the eprinomectin has no stress on animals. The application of eprinomectin in sheep indicated that on the 7th day 2(33%) sheep were recovered, while on 14th day 4(66%) sheep were recovered. It was concluded that parasitic infestation in sheep is of considerable significance in Bahawalpur division, whereas eprinomectin has a beneficial effect by improving animal health and reducing parasitic load.

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MAR, RAG, and AR designed and conceived the study, carried out the research, analyzed the data, wrote the manuscript. KM, AQ, MZ, SA, RS, EMA, and MSE, funding, resources, supervised, investigation, critically reviewed and revised the manuscript.

Key words
Parasites, Prevalence, Eprinomectin, Sheep, Hematobiochemical changes, Oxidative stress

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INTRODUCTION

Pakistan is an agricultural country and nearly 62% population of the country lives in the villages and is dependent on agriculture. The livestock sector contributes almost 60.6% to the agriculture share and 11.7% towards the national GDP of Pakistan (Anonymous, 2023). Livestock plays a pivotal role in the rural economy because almost

35-40 million population in the villages are connected to livestock and getting 30-40% of their income. Most families have 5 to 6 or more sheep or goats. The sheep population is projected to 32.4 million (Anonymous, 2023). The main health concern of sheep in rural areas is parasitic infestation, which includes endoparasite and ectoparasite (Qamar and Alkheraije, 2023). Parasitic infestation results decrease in weight gain, poor body condition, decrease in birth weights and difficult in lambing (Arsenopoulos *et al*., 2021). The grazing animals may become vulnerable to further health issues due to parasitic infection which ultimately causes death in animals (Mir *et al*., 2016). The poor health practices and low productivity in animals are related to decrease work capability, retarded growth and increase in expenditures for parasitic treatment and disease control (Tiele *et al*., 2023).

Parasitism is one of the major problems both in animals as well as humans (Tiele *et al*., 2023). In developing countries especially in Pakistan, ecto and endo parasitism are one of the major causes of economic losses with their prevalence of 30-60% in livestock (Muhammad *et al*., 2021; Ghafar *et al*., 2020). Two main endo-parasites are trematodes and nematodes which cause serious health issues and reduce small ruminant productivity via death, morbidity, and treatment costs, that decreases profit by 15% while body weight up to 50% (Tumusiime *et al*., 2022). The primary parasites that cause considerable economic losses and have an effect on national economy are small ruminant ectoparasites such as ticks, lice, flies, and bugs that spread throughout the animal population affecting the quality of the meat, milk, and leather produced (Muhammad *et al*., 2021; Seyoum *et al*., 2015). Similarly, Iqbal *et al*. (2015) reported that Ecto-parasites in sheep and goats cause significant economic losses by affecting skin conditions, quality of the meat (Asín *et al*., 2023; Strydom *et al*., 2023). The helminth infestations result in disruptions of blood chemistry and metabolic mechanisms in ruminant (Van-Wettere *et al*., 2021). Eventually, helminthiasis seriously reduces these animals' capacity for reproduction, productivity and it can even cause the death of lambs (Khan *et al*., 2021). The Pakistani livestock business has major obstacles due to parasitism, which is particularly detrimental to the development of a successful sheep industry (Batool *et al*., 2022).

Parasitic chemotherapy is the best way to treat helminth infection, though treatment is expensive and resistance to drugs has developed in major parasite species (Arsenopoulos *et al*., 2021). There is increased recognition of the need for an integrated approach to control parasites in sheep to maintain animal health, production and welfare throughout the world (Arsenopoulos *et al*., 2021). Sustainable parasitic control requires a topical and transdermal drug strategy to minimize the pressure for parasitic resistance and adaptation (Batool *et al*., 2022). Therefore, parasitic control requires a new strategy to minimize the pressure for parasitic infestation. Keeping in view the importance of anthelmintic resistance and the advantages of topical drug application, this study has been designed to investigate the clinico-epidemiological investigation of ectoparasites and endoparasites and the effect of eprinomectin to parasitic control in sheep in south Punjab.

MATERIALS AND METHODS

Study area

The study area for investigation of endoparasites and ectoparasites was Division Bahawalpur including its Tehsil Ahmedpur, Yazman, Khairpur (Tamewali), Hasilpur and Bahawalpur city.

Selection of animals

First of all, information was taken on a prescribed proforma about animal data (age, sex, breed and parturition etc.), disease history, area of grazing, digestion problem, and deworming. In most of the animals, the feeding practice included grazing on seasonal pastures. Meanwhile, house management including flooring and the health of animals can be observed. Animal with poor health practices, neglected treatment, poor feeding and housing management were included for this study after careful examination. The targeted number of animals was 320, which were considered for collection of fecal (160) and skin-scraping (160) samples. A total of 32 fecal samples and 32 skin-scraping samples were collected from each Tehsil of Bahawalpur.

Collection of samples

Samples were directly collected from the rectum by passing the index finger covered with disposable glove. About 10 grams per animal of fecal sample was collected and preserved in a polythene bag which was transported to the medicine laboratory for further examination. The scraping samples were collected with the help of scalpel blade and handle. The scraping samples were preserved in ethanol (5%) container and later transported to lab for further examination under microscope.

Processing and examination of samples

The collected fecal samples were processed by direct smear and floatation methods to observe under a microscope in the Medicine Lab in the Department of Clinical Medicine and Surgery, The Islamia University of Bahawalpur. Briefly a small amount of the fecal material

and a drop of water was put on a clean glass slide. After the mixing the formation, the large fecal particles were removed with the help of forceps and a cover slip was placed. The smear was examined under low power objective of microscope to detect the ova. Each sample was tested by direct smear method and the fecal samples that found negative in direct smear method were proceed by simple salt floatation method.

Whereas, some skin-scrapping samples were possible to be identified with the naked eye, while the remaining samples were observed under the microscope. Briefly, equal amount of skin scrap material and a drop of KOH was put on the middle of slide. KOH and scrapings were mixed properly with the help of needle to make a homogenous mixture and allow without interruption for 5 min. After the application of cover slip, the smear was examined under the microscope for the presence of parasite.

Preparation of eprinomectin

The topical eprinomectin formulation was prepared according to a commercially available preparation (Azone Laurocapram: 0.1ml+ Oleic Acid: 0.3ml+ Propylene Glycol: 0.4ml+ DMSO: 2ml+ 95% Ethanol: 2.2ml+ Eprinomectin @ 1 mg/kg body weight). One preparation of 5ml was prepared for each animal.

Therapeutic trial of eprinomectin

For the present study, twelve animals of both sexes including male and female were randomly divided into health groups (n= 6) and diseased groups (n= 6) positive for ecto-endo parasites. It was confirmed none of the animals was treated with antiparasitic products within six weeks of the start of the study. The formulation of eprinomectin 1 mg/kg (Rostang *et al*., 2019; Ballent *et al*., 2022) was administered once on day 1st topically (pour-on) along the back line, starting from the withers to the tail head. The animal health status was monitored throughout the study. No adverse reactions were observed at the site of application after treatment.

Collection and processing of blood samples

The blood samples of small ruminants were collected through the Juglar vein in EDTA-coated and gel-coated tubes from both groups on 1st day and 14th day and then shifted to laboratory for complete blood count and serum biochemistry. The blood analysis was performed by using a hematological analyzer (BK-5000VET, Bio-base industry Shandong Co., Ltd. China) at the Central Diagnostic Laboratory Complex, IUB. The serum biochemistry was performed with the help of commercially available kits by following the manufacturer's instructions.

Statistical analysis

The Data was analyzed by using SPSS software. Prevalence data was analyzed by using Mental Haensel chi-square and odd ratio. The blood and serum data were analyzed by one-way ANOVA. Whereas, $P<0.05$ was considered statistically significant.

Table I. Prevalence of ectoparasites in sheep.

Parameters	Total	No +ve (Prevalence %)	95% CI	Odd ratio/ P-value
Area wise				
Bahawalpur	32	14 (43.75%)	27.48 to 61.11	Mantel-Haenszel chi-sq P = 0.926
Ahmedpur	32	10 (31.25%)	17.09 to 48.67	
Hasilpur	32	9 (28.12%)	14.67 to 45.38	
Khairpur	32	7 (21.8%)	10.10 to 38.55	
Yazman	32	8 (25%)	12.34 to 42.01	
Total	160	48 (30%)	23.28 to 37.44	
Sex wise				
Male	74	22 (29.7%)	20.16 to 40.85	Odds ratio = 0.98 [recipro-cal = 1.02]
Female	86	26 (30.23%)	21.24 to 40.54	
Total	160	48 (30%)	23.28 to 37.44	
Age-wise				
Suckler	30	04 (13.33%)	4.38 to 29.10	Mantel-Haenszel chi-sq P = 0.236
Adult	98	36 (36.73%)	27.64 to 46.61	
Old	32	08 (25%)	12.34 to 42.01	
Total	160	48 (30%%)	23.28 to 37.44	
Species wise				
Ticks	70	30 (42.8%)	31.67 to 54.63	Mantel-Haenszel chi-sq P = 0.034
Lice	56	10 (17.8%)	9.44 to 29.52	
Mites	34	8 (23.5%)	11.57 to 39.84	
Total	160	48 (30%)	23.28 to 37.44	

RESULTS

Prevalence of ectoparasites in sheep

The prevalence of ectoparasites in sheep according to area, sex, age and parasitic species-wise are shown in Table I. There was no significant ($P>0.05$) difference in area-wise, sex-wise and age-wise determination. According to our study, the overall prevalence of ectoparasites in sheep area-wise was 30%, highest in the area of Bahawalpur (43.75%) then Ahmedpur (31.25%), Hasilpur (28.12%), Yazman (25%), and lowest in Khairpur (21.8%). According to sex wise prevalence of ectoparasites in sheep was greater in females (30.23%) than males (29.7%) but the difference was not significant. The age-wise

prevalence of ectoparasites in sheep was highest in adults (36.73%), old animals with 25% and lowest in sucklers (13.33%). There was significant (P<0.05) difference in parasitic species-wise prevalence. According to species wise, ticks' prevalence (42.8%) was significantly (P<0.05) higher than lice (17.8%) and mites (23.5%). The collected ectoparasites of sheep are shown in Figure 1.

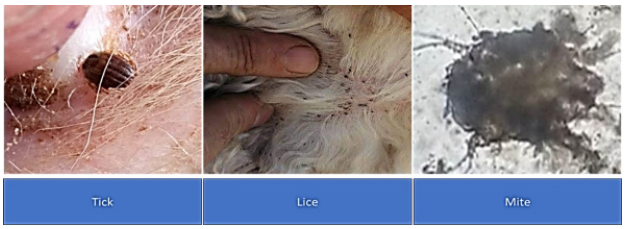

Fig. 1. Ectoparasites of sheep.

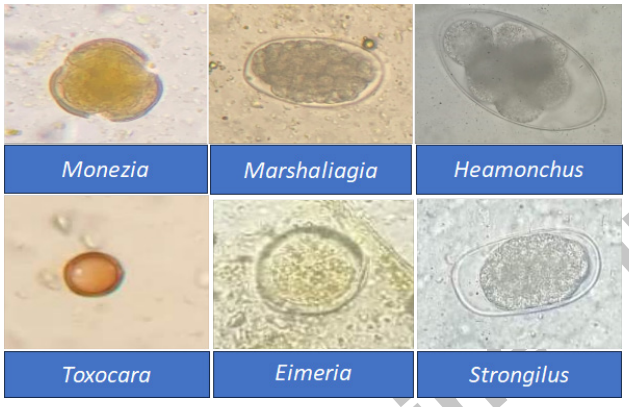

Fig. 2. Endoparasites of sheep.

Prevalence of endoparasites in sheep

The results show the prevalence of endoparasites in sheep according to area-wise, sex-wise, age-wise and parasitic-species-wise in Table II. There was no significant (P>0.05) difference in area-wise and parasitic-species prevalence. According to our study, the overall prevalence of endoparasites in sheep area-wise was 42.5%, highest in the area of Hasilpur (56.25%) then in Bahawalpur (46.8%), Ahmedpur (43.75%), Yazman (34.3%) and lowest in Khairpur (31.25%). According to sex wise prevalence of endoparasites in sheep was significantly (P<0.05) greater in females (47.4%) than males (35.4%). According to age, older age animals' prevalence was significantly (P<0.05) higher than others. Age-wise prevalence of endoparasites was highest in older age (68.88%), than adults (36.47%) and sucklers (20%). When we observed the prevalence of endoparasites in sheep according to parasitic-species wise, animals were more infected with *Toxocara* (45.9%) than *Monizia* (44%), *Emaria* (43.3%), *Strongylus* (42.9%), *Marshliagia* (38.9%) and lowest prevalence of *Haemonchus* (36.4%) was observed (Fig. 2).

Table II. Prevalence of endoparasites in sheep.

Parameters	Total	No +ve (Prevalence %)	95% CI	Odd Ratio/ P-value
Area wise				
Bahawalpur	32	15 (46.8%)	30.24 to 64.06	Mantel-Haenszel chi-sq P = 0.193
Ahmedpur	32	14 (43.75%)	27.48 to 61.11	
Hasilpur	32	18 (56.25%)	38.89 to 72.52	
Khairpur	32	10 (31.25%)	17.09 to 48.67	
Yazman	32	11 (34.3%)	19.58 to 51.88	
Total	160	68 (42.5%)	35.01 to 50.26	
Sex wise				
Male	65	23 (35.4%)	24.51 to 47.54	Odds ratio = 0.61; [recip-rocal = 1.64]
Female	95	45 (47.4%)	37.48 to 57.41	
Total	160	68 (42.5%)	35.01 to 50.26	
Age-wise				
Suckler	30	06 (20%)	8.53 to 37.03	Mantel-Haenszel chi-sq P = 0.003
Adult	85	31 (36.47%)	26.77 to 47.08	
Old	45	31 (68.88%)	54.34 to 81.07	
Total	160	68 (42.5%)	35.01 to 50.26	
Species wise				
Toxocara	37	17 (45.9%)	30.50 to 62.00	Mantel-Haenszel chi-sq P = 0.405
Strongylus	28	12 (42.9%)	25.68 to 61.44	
Emaria	30	13 (43.3%)	26.63 to 61.27	
Haemon-chus	22	8 (36.4%)	18.53 to 57.59	
Marshliagia	18	7 (38.9%)	18.86 to 62.25	
Monizia	25	11 (44%)	25.74 to 63.58	
Total	160	68 (42.5%)	35.01 to 50.26	

Blood parameters variation in sheep

Before the start of the trial, on the day first complete blood count (CBC) report of sheep among healthy and infected animals is shown in Table III. According to the results white blood cells (WBC), granulocytes (GR), red blood cells (RBC), hemoglobin (HBG), hematocrit (HCT) and mean corpuscular volume (MCV) was significantly (P<0.05) different between healthy and infected sheep. While other parameters were same (P>0.05). We observed that WBC, GR absolute value was significantly (P<0.05) higher in infected sheep while RBCs, HB, HCT and MCV were higher in healthy animals.

Table III. Blood parameters variation in sheep.

Groups/ Parameters	1st Day		P value	14th Day		P value
	Healthy	Infected		Healthy	Treatment	
WBC (10^3/µl)	5.66±0.11	12.53±0.93	0.002	4.83±0.20	6.08±0.20	0.007
LYM# (%)	5.49±0.03	6.03±0.25	0.189	5.36±0.26	5.13±0.10	0.344
GR# (%)	0.003±0.00	0.016±0.00	0.041	0.01±0.00	0.01±0.00	1.000
RBC (10^6/µl)	4.06±0.07	2.79±0.23	0.008	4.04±0.02	4.35±0.18	0.278
HB (g/dl)	10.66±0.34	8.10±0.26	0.001	10.90±0.05	9.95±0.25	0.039
HCT (%)	35.33±0.88	29.66±1.40	0.033	35.66±1.45	34.33±1.05	0.486
MCV (fL)	24.4±0.27	22.9±0.39	0.039	25.76±0.60	25.33±0.27	0.469
MCH (pg)	32.9±0.17	32.06±0.57	0.362	34.56±0.35	33.60±0.59	0.322

WBC, white blood cells; LYM, Lymphocytes; GR, granulocytes; RBC, red blood cells; HBG, hemoglobin; HCT, Hematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin.

During the trial, after the topical administration of the eprinomectin on the 14th day, the CBC report of sheep among healthy and infected animals is shown in Table III. According to results WBC and HB were significantly ($P<0.05$) different between healthy and treatment groups, while other parameters were non-significant ($P>0.05$).

Serum biochemical changes in sheep

After the administration of eprinomectin on 14th day the serum biochemistry analysis of healthy and Treatment group was performed, including total protein (TP), albumin (ALB), glutamic-pyruvic transaminase (GPT), and serum glutamic-oxaloacetic transaminase (SGOT) as shown in Table IV. According to the results, all parameters were same ($P<0.05$) between healthy and treatment groups. These results indicated that the eprinomectin has no stress on animals and other physiological changes in sheep.

Table IV. Serum biochemical changes in sheep.

Groups/ Parameters	Healthy	Treatment	P-value
TP (g/dl)	9.0±0.00	10.0±0.57	0.272
ALB (g/dl)	3.14±0.00	3.26±0.08	0.334
SGPT (IU/L)	32.5±7.5	27.3±0.66	0.428
SGOT (IU/L)	134.0±6.0	110.0±9.5	0.164

TP, total protein, ALB, albumin, GPT, glutamic-pyruvic transaminase, SGOT, serum glutamic-oxaloacetic transaminase.

Therapeutic efficacy of eprinomectin in sheep

The animals were observed after the treatment of eprinomectin in sheep on 7th and 14th days. The results indicated that on the 7th day 33% sheep were recovered, while on the 14th day 66% sheep were recovered as given in Table V.

Table V. Therapeutic efficacy of eprinomectin.

Days/ Animals	Sheep recovered (n=6)	Efficacy
Day 7th	2	33%
Day 14th	4	66

DISCUSSION

In this study, ecto and endoparasites infestation was prevalent in all tehsils of Bahawalpur, South Punjab. Among ectoparasitic infestation, ticks are found more prevalent than other ectoparasite. Ticks are recognized as important ectoparasites because, tick transmits diseases from one animal to another and modulate the immune system of the hosts (Ali *et al*., 2016). In Bannu KPK, ectoparasites infestations were 7.8, 15.2 and 6.8% for ticks, lice and mites, respectively in sheep. The prevalence of endoparasites was 87.4% in sheep, while the most common endoparasites were *Haemonchus*. *Trichurus*, Strongyloid, Coccidia, *Fasciola* and Trichostrongyloide (Khan *et al*., 2015). Shah *et al*. (2021) reported that prevalence of GI parasites was 73.9% in sheep in Central Zone of Khyber Pakhtunkhwa. A higher prevalence of ectoparasites was observed in young and female sheep. The prevalence of endoparasites (48.43%), and ticks (39.58%) were prominent in sheep. Yet, the association of extrinsic factors like tehsils with the prevalence was not significant in sheep population (Batool *et al*., 2022). Previously, overall prevalence of gastrointestinal parasites was reported 32.6%, while prevalence was more in female than male. These results are in line with present study. The endoparasites identified in sheep included *Fasciola* (8.3%), *Haemonchus* (23.4%), and *Eimeria* (18.2%). Among variables like age, and sex were prominent (Rizwan *et al*., 2017) which is also congruent to our study.

Regarding endoparasite infestation, increased prevalence of *Haemonchus* (36.4%), *Strongylus* (42.9%) and *Monizia* (44%) is reported in sheep in our study as compared to 29.58, 7.5 and 5.4%, respectively by Mehmood *et al*. (2013). They identified similar GIT parasites, including *Haemonchus*, *Strongylis, Trichuris* and *Monezia*. Breed, age, location, and the frequency of monthly tick infections are significant variables (Ali *et al*., 2016).

In Andhra Pradesh India, an overall prevalence was 70.6% in sheep, which include strongyles, (52.2%), and coccidia (3.8%). The tick infestation was 17.6% in sheep (Rajeshwari *et al*., 2023). In Ethiopia, it is reported that prevalence of ectoparasite in sheep was 27%, but the most common ectoparasite were lice (31.7%), tick (19%) and mite (12%). The statistical analysis indicated that sex and age were important factors significantly to ectoparasite infestation in sheep (Kasim *et al*., 2019). Feyisa (2021) reported an overall 66.5% infestation of ectoparasite with ticks (31%), followed by lice 15.5%, and mange mites 0.5%. In Italy, prevalence of endoparasites at flock level was 18-50% in sheep under mountain farming conditions (Lambertz *et al*., 2018). These differences in prevalence rate from present study may be due to different geographical location and difference in farming practice.

The hematological profile of infected animals revealed marked decrease in hemoglobin posing anemia while slight increase in total leukocyte count and eosinophils were also recorded (Khan *et al*., 2015). These results of hematological variations are in line to our findings in sheep. Anti-parasitic therapy to treat animals infested with parasitic infestation by using eprinomectin topically is of greater importance because it causes no stress to animals as indicated by liver parameters studied in our research were not changed in healthy and treatment groups.

Parasitic diseases cause productivity losses in dairy animals, with economic effects that can be reduced using anthelmintic therapy (Ballent *et al*., 2022). The most successful treatment for helminth infections is anthelmintic chemotherapy, yet it is a costly procedure and drug resistance has emerged against parasitic infestation in animals. Despite their benefits, anthelmintics used in dairy animals cause public health and food safety concerns due to the unwanted presence of drug residues in milk (Tsiboukis *et al*., 2013). Macrocyclic lactones (MLs) are anthelmintic drugs with endo-ectoparasiticidal activity (Rostang et al., 2019). Since injectable MLs are not approved for use in lactating animals, so eprinomectin is considered a first-line treatment option because it has less milk withdrawal period. Administration of eprinomectin via the topical route through pour-on is a new method and is long-lasting but it also originates less exposure to the drug (Bordes *et al*., 2022). In Argentina, eprinomectin is commercially registered as pour-on formulation for controlling ticks, because drug residues in milk are below the maximum level of residues (Ballent *et al*., 2022). Mao *et al*. (2019) reported that eprinomectin is a promising formulation for the treatment of endo and ectoparasites. Our results indicate that on the 7th day 33% of sheep were recovered while on the 14th day, 66% of sheep were recovered from parasitic infestation. Eprinomectin is a broad-spectrum endectocide compound and can be used to control gastrointestinal nematodes as well as ectoparasites (Imperiale *et al*., 2006).

CONCLUSION

It was concluded that gastrointestinal helminths and ectoparasites in sheep are of considerable significance in the Bahawalpur division, whereas eprinomectin has beneficial effect by improving animal health and reducing parasitic infestation.

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IRB approval

The research was approved from Institutional Review Board of Islamia University of Bahawalpur, Bahawalpur.

Ethical approval

The experiments were conducted in accordance with ethical guidelines and principles established by the Institutional Animal Ethics Committee, and approved by Office of Research, Innovation, and Commercialization, Islamia University of Bahawalpur. The consent of farmers was taken before collecting the samples from animals.

Statement of conflict of interest

The authors have declared no conflict of interest.

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