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EPIDEMIOLOGY, SERODIAGNOSIS AND THERAPEUTIC STUDIES ON OVINE NEMATODES AT DISTRICT LORALAI, BALOCHISTAN, PAKISTAN

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ABSTRACT

In present research study, three experiments were launched during January 2011 to December 2011. Experiment I, Epidemiology: A total 1200 faecal samples (100 per month) were collected from farmers Bibrik-sheep (either sexes, 1-5 years old) and analyzed for nematodes prevalence. Experiment II, Sero-diagnosis: The sera samples of healthy and nematodes infected sheep were analyzed through ELISA. Experiment III, Therapeutic trial: comparative efficacy of herbal and synthetic anthelmintics was determined. Results showed that overall 40.25% Bibrik-sheep were infected with nematodes. Five nematode genera were recorded with higher prevalence of *Haemonchus* (10.42%) followed by *Strongyloides* (8.75%), *Trichostrongylus* (7.33%), *Nematodirus* (6.92%), and *Trichuris* (6.83%). The older age ewes presented higher nematodes prevalence. These five genera of nematodes were prevalent throughout the year. However, two peaks of nematodes prevalence i.e. March/May and August/December 2011 were observed. The level of nematodes infection was mostly low (< 2000 EPG) and did not impair the sheep productivity seriously. *Haemonchus* and *Trichuris* positive samples, based on coprological examination indicated 100% positive sensitivity for each genus of nematode by the ELISA, based on crude somatic antigen, while based on excretory antigen, showed lower (92%) sensitivity. The higher (86-100%) reduction of EPG was recorded in sheep treated with Ivermectin, while lower (68-96%) with Atreefal deedan.

Key words: Sheep, Nematodes, Prevalence, ELISA, Cold environment, Herbal anthelmintics

INTRODUCTION

The internal parasites are major threat to livestock production throughout the world. Khan *et al*. (1988) reported 100% sheep in upland Baluchistan get infected with internal parasites. Razzaq *et al*. (2002) recorded overall internal parasitic infection in 93% sheep and 80% in goats at Asghara valley district Ziarat. Pedreira *et al*. (2006) recorded 100% prevalence of nematodes infecting sheep in Galicia (NW Spain). A common feature of gastro-intestinal parasites increased the loss of endogenous protein, which leads to retarded growth and some times heavy infection may cause mortality.

Several factors are known to determine the epidemiological pattern of the associated disease conditions. These include weather conditions, husbandry practices, and physiological status of the animal (Wall *et al*. 2004). The control of nematodes for the past thirty years has relied mainly on the use of chemotherapeutic agents. These compounds have been very successful but the development of anthelmintic resistance in different nematodes was reported in various countries. Recent surveys in the developing countries have identified many plants that have the potential to be used as anthelmintics (Jabbar *et al*. 2007). However, majority of the evidences reported in ethno-veterinary sources are based on observations, instead of proper experimentation. Based on their traditional uses, many plants have been validated scientifically for their anthelmintic properties (Iqbal *et al*. 2006). Therefore, the present study is accomplished to investigate epidemiological and sero-diagnosis of major gastro-intestinal nematodes infection in sheep. In addition, comparative efficacy of synthetic and herbal medicines was also determined against these nematodes.

MATERIALS AND METHODS

1. Prevalence of sheep nematodes (host-parasite and climate relationship).

a. Prevalence of nematodes in sheep: A total 100 Bibrik-sheep (either sexes and 1-5 years old) were selected from the surroundings of district Loralai (with the history of no de-worming for the last 3-4 months) to determine the prevalence of major nematodes. All the sheep were ear tagged before the initiation of experiment for proper maintenance of record through out the experimental period. Faecal samples were collected from all selected sheep, each month for one year (January 2011 to December 2011). Each faecal sample was analyzed for identification and counting of Egg/Larvae as Per Gram (EPG/LPG) faeces with McMaster technique (Urquhart *et al* 1996).

b. Metrological data: The weather data regarding rainfall, relative humidity and temperature during the complete year 2011 were obtained from Arid Zone Research Centre (PARC), Quetta for district Loralai at Balochistan, Pakistan. These climate factors were correlated with the prevalence of major nematodes of sheep.

2. Sero-diagnosis of *Haemonchus contortus* and *Trichuris ovis* in sheep

a. Collection of sera samples: In continuation of first experiment, the blood samples (5 ml, each) were collected from sheep (n=1000 from each zone), irrespective of the fact either positive or negative for *T. ovis* and *H. contortus* by faecal examination. After faecal analyses, the same sheep sera samples selected/separated as negative (n=300), and positive (infected) with *Haemonchus contortus* (n=200) and *Trichuris ovis* (n=200) for further comparision on ELISA.

b. Negative and positive control sera: The blood samples were collected on first day of life from three newly born lambs (not exposed to parasitic infection) from AZRC Range-Livestock Research Station, district Ziarat, Pakistan as negative control sera. The natural positive sera of 30 sheep were collected from Quetta abattoir. Initially blood samples for sera were collected and then confirmed harbouring nematodes during examination of gastro-intestinal tract of sheep after slaughtering.

c. Collection and isolation of nematodes: Adult *Haemonchus contortus* were collected from the infected abomasae and *Trichuris ovis* from caeca of these sheep form Quetta Slaughter-house. The identification of these nematodes was also confirmed using standard keys as described by Soulsby (1982).

d. Preparation of Crude somatic and Excretory-secretory antigen: The Crude-somatic and Excretory-secretory antigens of *Haemonchus* and *Trichuris* were prepared as described by Mir *et al.* (2008) and Prasad *et al.* (2008). Protein concentration of crude somatic and excretory-secretory antigens was determined by Lowey *et al*. (1951).

e. Protocol for ELISA test: The sera samples were analyzed through ELISA as described by Mir *et al*. (2008), Ahmad *et al*. (2011) and Arunkumar (2012).

3. Field/community therapeutic trial: The comparative efficacy of synthetic (Ivermectin) and locally manufactured herbal anthelmintic (Atreefal deedan) against sheep nematodes was determined. The nematodes infected sheep (n=90) were equally divided into three main groups A, B and C (n=30 sheep in each group). These nematodes infected sheep groups were kept as infected control (Group A), treated with Ivermectin (Group B) and Atreefal deedan (Group C). Each group of sheep from B and C were further comprised of sub-groups (n=6) being infected with five genera of nematodes i.e., *Strongyloides, Trichostrongylus, Haemonchus, Trichuris* and *Nematodirus* between 1500-2000 EPG levels (Table 1). Faecal samples from all these experimental sheep were collected pre treatment i.e. on day 0 followed by post treatment on day 3^{rd}, 5^{th}, 7^{th}, 10^{th} and 14^{th} for counting EPG as described by Urquhart (*et al*. 1996). Efficacies of the anthelmintic were calculated as per formula described by Ali (2001).

$$\text{Efficacy \%} = \frac{\text{EPG before treatment - EPG post treatment}}{\text{EPG before treatment}} \times 100$$

Statistical analysis: The data collected from this study were analyzed by using GraphPad Prism-5 computer statistical package. The prevalence of different nematodes was analyzed through Chi-squire test, one way and some time two way analyses of variance. In addition, "Dunn's Multiple Comparison Test" was also performed when different groups of sheep were compared when infected with different genera of nematodes and months-wise prevalence.

RESULTS AND DISCUSSION

1. Prevalence of sheep nematodes (host-parasite and climate relationship)

a. Area/breed wise nematodes prevalence: The Bibrik-sheep faecal analysis showed overall 40.25% prevalence of nematodes during January to December 2011 from district Loralai. Five genera of nematodes were recorded with higher prevalence of *Haemonchus* (10.42%) followed by *Strongyloides* (8.75%), *Trichostrongylus* (7.33%), *Nematodirus* (6.92%), and *Trichuris* (6.83%) during the study period. Similarly *Haemonchus* also showed higher EPG followed by rest of the nematodes (Table 2). Statistically significant differences ($P<0.05$) were observed between the prevalence of different genera of nematodes and their EPG levels. Mushtaq and Tasawar (2011) recorded higher prevalence 46.33% in sheep at Southern Punjab, Pakistan and Gadahi *et al*. (2009) recorded even higher 63.50% endo-parasites prevalence in sheep around Rawalpindi and Islamabad. During another study, Pedreira *et al*. (2006) recorded heighest prevalence (100%) of nematodes infecting sheep in Galicia (NW Spain). The low prevalence of nematodes in present study might be due to dry environmental conditions as drought prevalent in the study area. Radostits *et al*. (1994) mentioned that the out-breaks are more likely to occur under wet hot conditions. Mushtaq and Tasawar (2011) observed highest infection in Kacchi breed than Lohi breed and recorded prevalence percentage of *Haemonchus contortus* and *Trichuris*

globulosa as 6.50% and 5.73%, respectively at Southern Punjab, Pakistan.

b. Age wise prevalence of sheep nematodes: The prevalence of different nematodes infection was recorded in all five age groups of sheep at Loralai District. Five years older Sheep showed higher nematode prevalence followed by three, four and one year old age groups. However, sheep less than three years age group presented higher EPG (Table 2). The difference in nematode prevalence of different age groups was statistically significant (P<0.05). Qamar and Maqbool (2012) revealed that prevalence was higher (40.31%) in sheep of less than nine months old than the animals above this age (33.08%). Abunna *et al*. (2009) stated that there was no significant difference between age and month.

c. Sex wise prevalence of sheep nematodes: To investigate the sex wise prevalence of nematodes, a total of 600 sheep of either sex were selected. The results showed that the prevalence of nematodes was higher in female (52%) than the male (28.5%), while mean EPG was higher in male than the female sheep (Table 2). The difference in the prevalence of nematodes and EPG in either sex groups were statistically significant (P<0.05)**.** Qamar and Maqbool (2012) was in view that animals of either sex were equally affected by the helminthes. Mushtaq and Tasawar (2011) pointed out that the prevalence of gastro-intestinal parasites was higher in male-sheep than female-sheep. Urquhart *et al*. (1996) stated that male is more susceptible than female due to androgen hormones. The females are more resistant to infection might be due to the stimulatory effects of estrogen on immune response, whereas the androgen have an opposite effect in males. The difference in sex-wise prevalence could be due to the fact that studies were conducted in different season and area.

d. Month wise prevalence of sheep nematodes and its relationship metrological data: In present study, an annual pattern of occurrence of sheep nematodes was also investigated at district Loralai. A total of 100 faecal samples were collected per month over a period of one year (January to December 2011) and analyzed. The results revealed that there were two peaks of nematodes prevalence i.e. March/May and August/December 2011 (Fig. 1). Similarly, two parallel peaks of these nematodes EPG were also recorded in this experiment. The statistical analysis showed significant (P<0.05) difference of nematodes prevalence during different months of the year (2011). However higher EPG varied among different nematodes. *Haemonchus contortus* showed higher EPG during November, *Strongyloides papillosus* and *Trichostrongylus colubriformis* during August, *Nematodirus battus* during March and *Trichuris ovis* during September. There was statistically significant (P<0.05) difference of sheep nematodes EPG during different months of the year.

The climatic data of district Loralai revealed that, the average humidity was between 15-35%. The average temperature was higher (36-37 ^{o}C) during May to August 2011. A total 130 mm rainfall received during 2011 at district Loralai. Higher rainfall was recorded during February (Table 3). The two peaks of prevalence of overall five nematodes genera during March/May and August/ December 2011 were directly related to favourable climatic conditions like rainfall (20-33mm), temperature (24-36 ^{o}C) and humidity (28-45%). Haemonchus was predominant among the nematodes at Loralai which might be due to hot climate that facilitate the infective larval development and subsequent infection to sheep. Different epidemiological factors, as also mentioned by Urquhart *et al*. (1996), were experienced in this study that the chronic haemonchosis develops during a prolonged dry season where re-infection is negligible, and when the pasture becomes deficient in nutrients. The clinical haemonchosis was observed in grazing lambs in late summer. Radostits *et al*. (1994) mentioned that the out-breaks are more likely to occur under wet hot conditions like hot conditions at Loralai in the present study.

2. Sero-diagnosis of sheep nematodes: The comparative sensitivity of immunological technique was determined with coprological examination in present study. The results revealed that the *Haemonchus contortus* and *Trichuris ovis* positive sera samples (200) based on coprological analysis were also indicated 100% positive sensitivity by the ELISA based on excretory-secretory antigen, while with crude somatic antigen based ELISA showed lower (92 %) sensitivity (Table 4). Surprisingly, the ELISA based on excretory-secretory antigen also indicated 127 (42%) and 119 (39.66%) positive samples of *Haemonchus contortus* and *Trichuris ovis* respectively out of 300 negative samples through coprological analysis. The same negative samples on crude somatic antigen based ELISA also showed 130 (43.33%) and 112 (37.33%) positive with *Haemonchus contortus* and *Trichuris ovis*, respectively (Table 7). Mir *et al*. (2008) also reported similar results of two antigens. They point out that the ELISA sensitivity based on excretory-secretory antigen was significantly higher (87.5%) compared to crude somatic antigen (72.22%). Almost similar finding were also reported by Prasad *et al*. (2008). The ELISA enables the detection of subclinical or early infection is very important (Lone *et al*., 2012). Hence serological diagnosis should be preferred because *anti-H. contortus* antibodies can be detected as early as one week post infection and thus can facilitate early chemotherapeutic intervention (Qamar and Maqbool, 2012).

3. Field/community therapeutic trial: The experimental sheep infected with five nematodes genera having almost similar EPG (1750) was selected as control-positive group (Group -A). The mean EPG, between 1500-2000, of these sheep were remained continuously recorded for five times during the study. The Ivermectin treated sheep (Group-C) showed 100% reduction in EPG being infected with *Haemonchus*, *Trichuris* and *Nematodirus* while lower (91% and 86%) reduction in EPG being infected with *Strongyloides* and *Trichostrongylus* respectively (Fig. 2 and 3). There was significant difference of reducing in EPG between pre and post treatment. Atreefal deedan (herbal anthelmintic) treated sheep (Group-C) showed mean reduction of EPG less than 500 on day 14th post treatment (Fig 4). The EPG was reduced between 68-96% that revealed effectiveness of this herbal product against selected nematodes. The results showed that there was a significant difference between pre and post treatment. Similarly Sheferaw and Asha (2010) also find out similar faecal egg count reduction like 98.3% with Ivermectin against gastro-intestinal nematodes of sheep. Mirhadi *et al*. (2011) also presented similar efficacy that the Ivermectin against *Nematodirus spathiger* reduced 99.1% faecal egg count. The traditional uses of different plants as anthelmintics were experimentally tested by different researchers to justify its usage. There is limited published literature available about herbal products used in the present study. However some related plants alone and with comparison to synthetic anthelmintics were used by different researchers and found effective against nematodes (Iqbal *et al*. 2006:Jabbar *et al*. 2007).

Table 1: Therapeutic experimental design

Groups (n=30 in each group)	Sub-groups (n=6 in each)	Treatment (Name, dose and composition)
A (Infected control)	*Strongyloides* *Trichostrongylus* *Haemonchus* *Trichuris* *Nematodirus*	Nil
B (Treated)	-do-	Ivermectin @ 20 ug/kg
C (Treated)	-do-	Atreefal deedan @ 10 g/5 kg body weight (composed of *Emblica officianalis, Terminalia bellerica, Terminalia chebula, Embelia robusta, Ipomoea turpethum, Saussurea lappa, Mallotus philippinensis, Lupinus albus, Artemisia absinthium, Darmina turki, Cascuta reflexa, Black salt, Brassica cernua, Citrullus colocynthis, Cyprus scariosus, Zingiber officinale,* liquid glucose and sugar)

Table 2. Prevalence of sheep nematodes at District Loralai

Nematodes/age/sex wise		Total infected (n=1200)	Prevalence	Mean ± SD of EPG
Nematodes	*Strongyloides*	105	8.75	691.53±51.88
	Trichostrongylus	88	7.33	724.78±155.11
	Haemonchus	125	10.42	1546.17±367.11*
	Trichuris	82	6.83	569.37±84.15
	Nematodirus	83	6.92	514.71±89.59
Age (Year)	1	80	33.33	1162.27±152.90*
	2	80	33.33	1052.27±219.00*
	3	103	42.92	532.49±89.33
	4	99	41.25	560.81±105.33
	5	121	50.42	641.45±124.55
Sex	Male	171	28.5	1478.82±180.23
	Female	312	52.0	1487.20±191.19
	Overall	**483**	**40.25**	

Table 3. Average humidity/temperature and total rainfall of district Loralai during 2011

Months	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Humidity (%)	35	44	28	22	15	21	31	45	41	20	27	17
Temp (^{o}C)	14	17	24	28	37	39	37	36	33	29	23	17
Rain (mm)	0	33	26	12	4	0	9	20	26	0	0	0

Table 4. Comparative sensitivity of ELISA and coprological examination for determination of *Haemonchus contortus* and *Trichuris ovis* infection in sheep.

Species of Nematodes	Total Sample examined	Coprological analysis	Excretory-Secretory Ag	Crude-somatic Ag
Haemonchus contortus	200	200 (100%)	200 (100%)	184 (92%)
Haemonchus contortus	300	- (0%)	127 (42%)	119 (39.66%)
Trichuris ovis	200	200 (100%)	200 (100%)	184 (92%)
Trichuris ovis	300	- (0%)	130 (43.33%)	112 (37.33%)

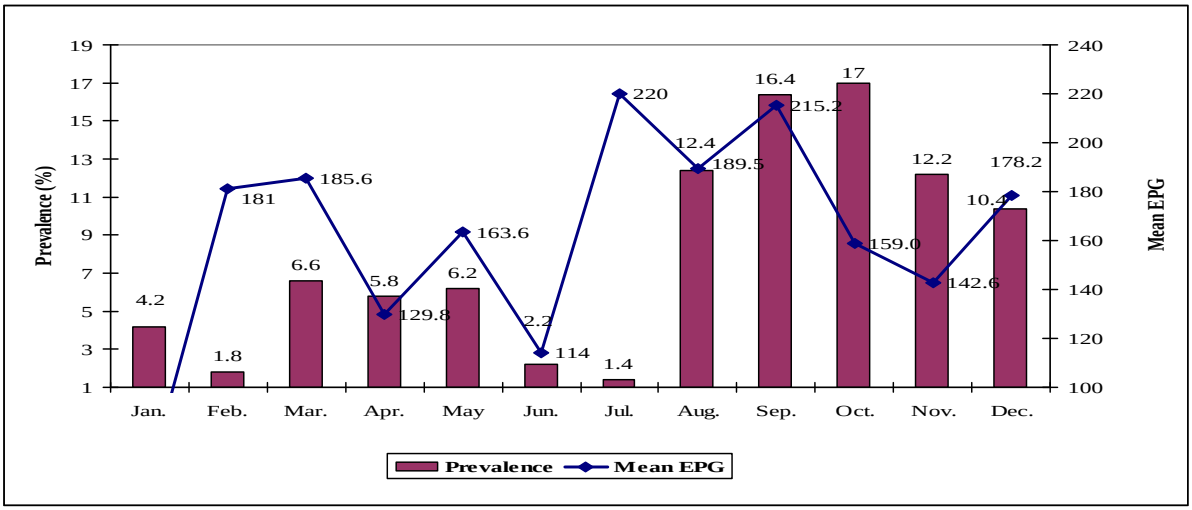

Fig 1. Month-wise prevalence and mean EPG of sheep nematodes at district Loralai

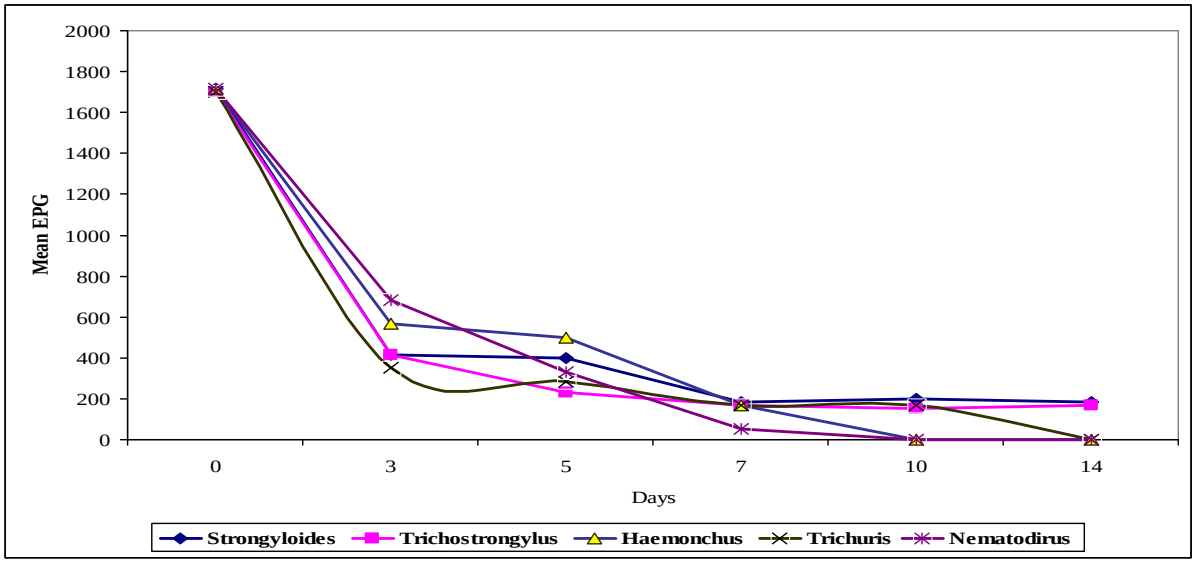

Fig 2. Mean EPG reduction of nematode infected sheep treated with Ivermectin

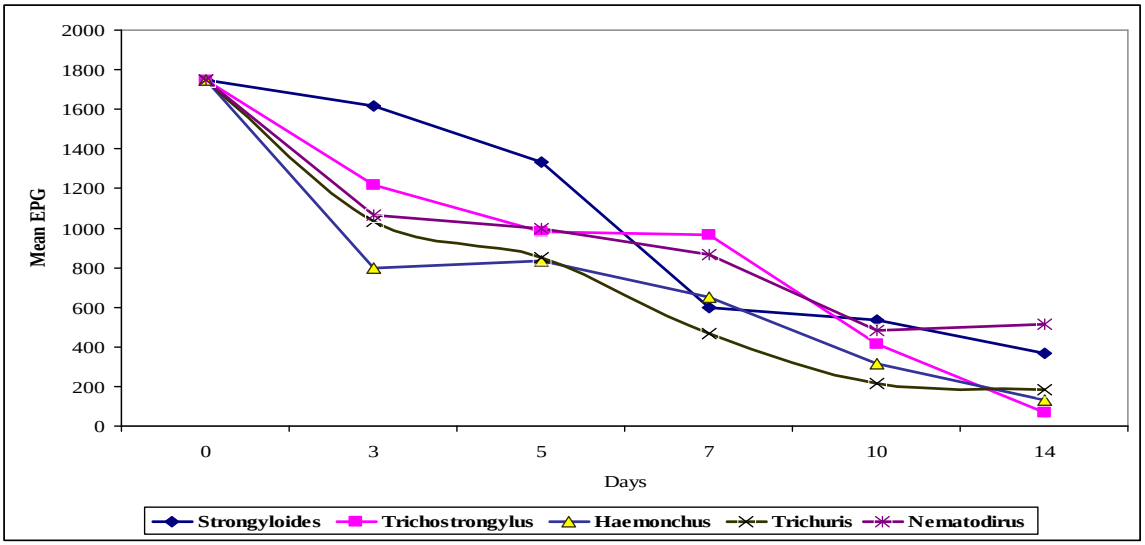

Fig 3. Mean EPG reduction of nematode infected sheep treated with Atreefal deedan

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