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EPIDEMIOLOGY AND THERAPEUTIC STUDIES ON NEMATODES INFECTION IN CATTLE AT DISTRICT QILLA ABDULLAH, BALOCHISTAN

M. Ramzan[1,2], N. Ahmad[2], K. Ashraf[2], K. Saeed[2], A. `Z. Durrani[2], S. Jan[3], Rafiuddin[1] and M. A. Khan[1]

[1]Livestock and Dairy Development Department Balohistan, Pakistan; [2]University of Veterinary and Animal Sciences Lahore, Pakistan; [3]Centre for Advanced Studies in Vaccinology and Biotechnology (CASVAB) University of Balochistan, Pakistan.
[*]Corresponding author Email:drmramadhan@yahoo.com

ABSTRACT

A study was conducted to assess the epidemiological and therapeutic control aspects of gastrointestinal nematodes of cattle at district Qilla Abdullah, Balochistan, from March 2012 to February 2013.A total of 600 fecal samples (50 samples per month) were examined during the study period. Fecal analysis showed overall higher nematodes prevalence as 27.99%. Among nematodes higher prevalence was recorded with infection of *Ostertagia* in cattle. Among age groups, cattle of ≤1 year of age presented higher nematodes prevalence than 1-2 years and >2 years age groups. Female cattle were infected higher than male animals with nematode parasites. The nematodes were prevalent throughout the year, however a peak infection was recorded during August and September. The therapeutic studies showed higher (89-100%) reduction of EPG in cattle calves treated with Ivermectin followed by Levamisole (88-100%) and Oxfendazole (86-100%).The present study was accomplished for a better understanding of gastrointestinal nematode infections under local climate and management system of the study area of Balochistan.

Key words: Gastrointestinal Nematodes, Cattle, *Hemonchus, Ostertagia, Trichostrongylus, Nematodirus, Trichuris,* Qilla Abdullah.

INTRODUCTION

Parasitism is of supreme importance in many agro-ecological zones and poses a serious threat to the livestock economy worldwide (Vercruysse and Claerebout 2001). Parasitic diseases are a major health concern in livestock production in many parts of Pakistan (Khan *et al*., 2010).Depressed feed intake, impaired tissue deposition, skeletal growth and economic losses are commonly seen in animals infected with parasites (Soulsby, 1982; Urquhart *et al*.1996; Radiostits *et al*. 2007). The economic losses associated with gastrointestinal tract parasites in cattle are universally accepted (Charlier *et al*. 2009). Different prevalence of helminthes has been reported in many countries and it range from 0.62 to 100% in domestic ruminants under various managemental conditions (Liu *et al*.2009;Pilarczyk *et al*. 2009; Siddiki *et al*. 2010; Hiko *et al*, 2011; Marufu *et al*. 2011; Yagoob *et al*. 2011). Similarly, studies in Pakistan have revealed widespread occurance of helminthes in our domestic animals and infection rates as high as over 90% are reported (Rhman *et al*.2009).Interaction of various risk factors influences the prevalence of gastrointestinal nematodes in food animals and include age, sex, season, use of anthelmintics and husbandry or management practices at the farm (Raza *et al*. 2007; Khan *et al*. 2009). A better understanding of the epidemiology and biology of parasites is essential for a successful parasite control program. A Sustainable control strategies complemented with effective anthelmintics and selective breeding for enhanced resistance are desirable for parasite control (Coppieters, 2009).

The climate of the certain areas of Balochistan is quite different from many tropical, subtropical and temperate regions of the world and arid areas of Pakistan. Information on the epidemiology of gastrointestinal nematodes of cattle from this region was not available. Keeping in view the scarcity of information and the importance of gastrointestinal nematodes in domestic cattle, the present study was accomplished for a better understanding of gastrointestinal nematode infections under local climate and management system of the study area of Balochistan.

MATERIALS AND METHODS

***Experiment 1: Epidemiology of G.I.T Nematodiasisin cattle*:** Study animals included were cattle of various age groups of either sex, maintained under different management practices. The present study was designed from March 2012 to February 2013, during which a total of 600 samples from gastrointestinal tract (50/month) were collected directly from the rectum of each animal in a polythene bags and were labeled with a permanent marker. These samples were shifted to the Disease Investigation Laboratory of Livestock Department, Quetta for isolation and identification of gastrointestinal

nematodes. The coprological examination was accomplished by direct Smear method, salt floatation method, McMaster egg counting technique and Baerman's technique. Animal population for this study was from private dairy units and household cattle maintained in different villages of district Qilla Abdullah.

Experiment 2: To Asses the efficacy of three commercially available anthelmintics: The efficacy of three group of anthelmintics i.e. Benzimidazole (Oxfendazole), Imidazothiazole (Levamisole) and Macrocyclic lactones (Ivermectin) available in the market were determined by Fecal Egg Count Reduction Test (FECRT). This method was used to see the effect of different anthelmintics and efficacy was calculated according to the method described in the World Association for the Advancement of Veterinary Parasitology (WAAVP) recommendations (Coles *et al*. 2006). A total of fifty (50) cow calves under one year of age naturally infected with gastrointestinal nematodes were included in the study. The study was conducted at a private cattle farms. The infected cow calves were divided into five groups, each comprised of 10 animals. The groups were maintained as a test group of cow calves C1, C2, C3, C4 & C5.Group C1 was given Oxfendazole, Group C2 was administered with Levamisole and C3 was injected with Ivermectin, animals in C4 group were kept as untreated positive control groups and animals in C5 group were kept untreated negative control groups (cow calves) respectively. A single dose of Oxfendazole (Oxafax) was administered @4.5 mg/kg body weight, Levamisole (Nilzan) was given @7.5 mg/kg body weight orally and Ivermectin (Ivomec) was injected @0.2 mg/kg body weight by Subcutaneous route. Egg per gram of feces (EPG) was determined by quantitative examination of feces by nematode egg counting technique on days 7Th, 14th and 28th post medication. Fecal samples from each animal was examined to determine the efficacy by the following equation (Coles *et al*. 2006).

$$\text{Efficacy \%age} = \frac{\text{EPG of feces before the treatment- EPG of feces after treatment}}{\text{EPG before treatment}} \times 100$$

Statistical Analysis: Data generated from this yearlong study were stored in Microsoft Excel 2013 and analyzed by GraphPad software Prism version 6 (GraphPad Software. Inc. San Diego, California, USA 9 www.graphpad.com). The prevalence of different nematodes was analyzed through the Chi-square test, one way and sometime two way analysis of variance. The efficacy of various treatments against nematodes was also analyzed by one way analysis of variance of each treatment group Individually.

RESULTS

Experiment 1: Epidemiology of G.I.T Nematodiasis in cattle:

a. Prevalence of nematodes in cattle: Fecal analysis showed overall higher prevalence (27.99%) for mixed gastrointestinal nematodes infection. Among these, *Ostertagia ostertagi* showed higher (9%) prevalence followed by *Haemonchus contotus* (8.33%), *Trichostrongylus colubriformis* (3%), *Nematodirus spathiger* (5.83%) and *Trichuris spp.* (2.11%) during the study period. Statistical significances ($P<0.05$) were observed between different nematodes prevalence and its EPG levels.

b. Age-wise prevalence of nematodes in cattle: The prevalence of five nematode infection of all age groups (< 1 year, 1-2 years and > 2 years) of cattle was recorded during 12 months .Cattle calves< 1 year showed higher prevalence (34%) followed by 1-2 years (29%) and above 2 years(21%). The cattle < 1 year of age presented higher EPG (274) count followed by 1-2 years (122) and > 2 year (70) of age groups. The difference in nematode prevalence in different age groups was not significant, however EPG level of nematodes in different age group showed significant differences ($P<0.05$).

c. Sex-wise prevalence of nematodes in cattle: The difference of nematodes prevalence in either sex was not statistically significant.

d. Month-wise prevalence of nematodes in cattle: Results revealed that there were two peaks of nematodes prevalence i.e. March/April and August/September 2012. The statistical analysis showed significant ($P<0.05$) difference in nematodes prevalence during different months of the year (March 2012 to February 2013). Similarly the peak of all five nematodes was recorded during August. However higher EPG were varied among the different nematodes. *Haemonchus contortus* showed higher EPG during August, *Ostertagia* during February and *Trichstrongylus colubroformis* during August, *Nematodirus spathiger* during March and *Trichuris* during September. There was statistically significant difference ($P<0.05$) between EPG Level and between different months of the year.

Experiment 2: Field Therapeutic Trials

Group C1: Nematodes infected cattle treated with Oxfendazole: Ten cattle calves, infected with nematodes having almost similar EPG level confirmed after fecal analysis, were selected as first treatment group (C1). Oxfendazole was given to all these infected cattle calves followed by analysis of fecal sample for reduction of EPG on day 0, 7th, 14th and 28th post treatment. The mean EPG was less than 5 on day 28th post treatment. The EPG

was reduced 86-100%which revealed effectiveness of Oxfendazole against selected nematodes. The result showed that there was significant difference between pre and post treatment EPG level.

Group C2: Nematodes infected cattle treated with Levamisole: The result showed that there was significant difference in reducing EPG post treatment.

Group C3: Nematodes infected cattle treated with Ivermectin: There was significant difference in reducing the EPG between pre and post treatment.

Group C4: Nematodes free/untreated positive control group cattle: All ten cattle were kept asnematodes free untreated positive control group, confirmed by fecal examination, at private dairy farm of district Qilla Abdullah. These cattle were household in-controlled condition by providing clean drinking water and feed. Afterwards four times (day 0, 7^{th}, 14^{th} and 28^{th}) fecal sampling and its analysis also confirmed them free of nematodes infection.

Group C5: Nematodes infected/untreated negative control group cattle: Ten cattle having GIT nematodes with EPG were selected as infected untreated negative control group. The mean EPG (228-468) of these cattle was continuously recorded four times.

Table 1. Nos. of fecal samples collection from cattle at district Qilla Abdullah

Season	Age	Private Farm		House-hold	
		Male	Female	Male	Female
Spring	<12 M	5	20	5	20
	1-2 Y	5	20	5	20
	>2 Y	5	20	5	20
Summer	<12 M	5	20	5	20
	1-2 Y	5	20	5	20
	>2 Y	5	20	5	20
Autumn	<12 M	5	20	5	20
	1-2 Y	5	20	5	20
	>2 Y	5	20	5	20
Winter	<12 M	5	20	5	20
	1-2 Y	5	20	5	20
	>2 Y	5	20	5	20
Total		**60**	**240**	**60**	**240**

Total Samples = 600 M= Months, Y= Years

Table 2. Prevalence of cattle nematodes at District Qilla Abdullah.

Parasites	Total Infected (n=600)	Prevalence (%)	Mean EPG
Haemonchus contortus	50	8.33	326^{**}
Nematodirus spathiger	35	5.83	172.83^{*}
Ostertagia ostertagi	54	9	343.66^{***}
Trichostrongylus colubriformis	18	3	151.5
Trichuris	11	1.83	119.33
Total	**168**	**27.99**	

Table 3. Age-wise prevalence with mean EPG of cattle nematodes at districtQilla Abdullah.

Age	No. of Samples collected	Positive	Prevalence %	Mean EPG
<12 M	200	68	34	208.40
1-2 Y	200	58	29	116.60
>2 Y	200	42	21	90.00
Total	**600**	**168**	**28**	

Table 4. Sex-wise prevalence with mean EPG of cattle nematodes at District Qilla Abdullah.

Sex	No. of Samples collected	Positive	Prevalence %	Mean EPG
Male	120	32	26.66	196
Female	480	136	28.33	210
Total	**600**	**174**	**29**	

Table 5. Month-wise prevalence (%) and mean EPG of cattle nematodes in District Qilla Abdullah.

Months	*H. contortus*	*N. spathiger*	*O. ostertagi*	*T. colubriformis*	*Trichuris*	Cumulative %
Mar-2012	3(292)	6(196)	25(612)	3(162)	1(98)	7.6(272)
Apr-2012	10(432)	14(194)	20(598)	1(132)	1(172)	9.2(306)
May-2012	5(476)	16(168)	7(192)	4(92)	2(88)	6.8(204)
Jun-2012	2(488)	7(176)	1(172)	2(172)	1(128)	2.6(227)
July-2012	5(492)	9(136)	1(132)	2(276)	1(32)	3.6(213)
Aug-2012	7(510)	20(222)	12(198)	22(298)	3(198)	12.8(285)
Sept-2012	15(362)	15(276)	12(372)	15(288)	12(232)	13.8(338)
Oct-2012	12(312)	6(184)	6(388)	7(178)	7(200)	7.6(284)
Nov-2012	10(286)	8(96)	1(490)	1(132)	4(190)	4.8(238)
Dec-2012	0(0)	1(228)	7(532)	1(88)	2(62)	2.2(234)
Jan-2013	0(0)	0(0)	9(200)	0(0)	1(32)	2(86)
Feb-2013	0(0)	0(0)	10(238)	0(0)	00)	2 (48)

Table 6. Mean EPG reduction and mean EPG reduction (%) of cattle treated with Oxfendazole.

Parasites	Pre-Treatment	Post treatment		
	Day 0	Day 7	Day 14	Day 28
Haemonchus contortus	212	8(96.22%)	4(98.11%)	0(100%)
Nematodirus spathiger	192	52(73%)	36(81.25%)	4(98%)
Ostertagia ostertagi	240	8(97%)	12(95%)	0(100%)
Trichostrongylus colubriformis	168	32(81%)	20(88%)	0(100%)
Trichuris	132	0(100%)	0(100%)	0(100%)

Table 7. Mean EPG reduction and mean EPG reduction (%) of cattle treated with Levamisole.

Parasites	Pre-Treatment	Post Treatment		
	Day 0	Day 7	Day 14	Day 28
Haemonchus contortus	232	48(80%)	12 (95%)	0(100%)
Nematodirus spathiger	200	18(91%)	11(95%)	4(98%)
Ostertagia ostertagi	264	24(91%)	8(97%)	0(100%)
Trichostrongylus colubriformis	188	5(97%)	0(100%)	0(100%)
Trichuris	92	12(87%)	6(94%)	3(97%)

Table 8. Mean EPG reduction and mean EPG reduction (%) of cattle treated with Ivermectin.

	Pre Treatment	Post Treatment		
Parasites	Day 0	Day 7	Day 14	Day 28
Haemonchus contortus	88	11(87.5%)	5(94%)	0(100%)
Nematodirus spathiger	84	16(81%)	10 (88%)	0 (100%)
Ostertagia ostertagi	176	40(97%)	3(98%)	0(100%)
Trichostrongylus colubriformis	68	20(71%)	4(95%)	0(100%)
Trichuris	44	11(75%)	8(82%)	0(100%)

DISCUSSION

The overall prevalence of gastrointestinal nematodes in cattle was 27.66% at district Qilla Abdullah during the study period (March 2012 to February 2013). There are various reports on the prevalence of gastrointestinal nematodes in various region of Pakistan. Khan *et al*. (2010) recorded almost similar overall prevalence (33.68%) in cattle in Toba Tek Singh, while Bilal *et al*.(2009) and Rafiullah *et al*. (2011) recorded higher prevalence (56.26%, 64.61%)in cattle at Toba Tek Singh and Peshawar, Pakistan, respectively. Moyo (2006) even recorded higher prevalence (100%) of gastrointestinal nematodes than the present study infecting large ruminants in Zimbabwe. The low prevalence of gastrointestinal nematodes in present study might be due to dry and cold environmental condition in the study area. Radostits *et al*. (2007) mentioned that the outbreaks are more likely to occur under wet hot conditions. In addition proper management and husbandry practices leads to low gastrointestinal nematodes infection, while the gastrointestinal nematodes infection are considered to be serious concern in grazing animals (Sanchez *et al*. 2004). Five genera of gastrointestinal nematode parasites were recovered from gastrointestinal tract during study period. Among these, *Ostertagia ostertagi* was highly prevalent (9%) in cattle followed by *Haemonchu scontortus* (8.33%), *Nematodirus spathiger* (5.83%) *Trichostrongylus colubriformis* (3%),and *Trichuris* (1.83%). Chanei *et al* (2012) reported the gastrointestinal nematodes species prevalence of *Ascaris* (57%), *Strongyles* (56.07%) and *Trichuris* (16.82%). Gastrointestinal nematodes species prevalence in cattle recorded by (Asif Raza *et al*. 2013) were *Toxocara vitulorum*, *Oesophagostomum radiatum*, *Haemonchus placei*and *Bunostomum phlebotomum.* The overall prevalence was 21% for these nematodes. Similarly (Mir *et al*. 2013) recorded the gastrointestinal nematodes in cattle were *Haemonchus* spp.(11.93 %), *Trichuris* spp. (5.16%), *Chabertia* spp. (4.83%). Laha *et al*. (2013) also recorded the prevalence of gastrointestinal nematode species as *Strongyle* spp.(65.96%) followed by *Strongyloides* spp. (25.13%), *Trichuris* spp. (13.08%) and *Nematodirus* spp.(2.61%).Difference in the incidence of different internal parasitic species in the present and other studies conducted in different locations may be due to different ecologies, temperature, management practices, forage and fodder availability. In the present study age-wise prevalence of gastrointestinal nematodes in cattle under 12 months showed higher prevalence (34%) followed by 1-2 years (29%) and> 2 years (21%). Cattle < 1 year of age showed higher EPG (274), followed by 1-2 years (122) and > 2 years (70) of age groups. Bilal *et al*.(2009) stated that cow calves between 1 to 6 months of age exhibited highest prevalence of gastrointestinal parasites as compared to the age group of 7 to 12 months. Swai *et al*. (2006) reported higher infection rates with nematodes in immature cattle as compared with adult cattle, while Biu *et al*.(2009) stated that ova/oocysts of gastrointestinal parasites of ruminants indicates the younger ruminants were more infected as compared to the older ruminants. Similarly Chanei *et al*. (2012) reported the higher prevalence of infection with GInematodes in calf (41.30%) than in young cattle (34.14%) and adult cattle (23.07%). Another study revealed (Asif Raza *et al*. 2013) that age-wise prevalence of different GI nematodes in calf like *Toxocaravitulorum* was 25.64% while in adult animal it was 8.19%, *Oesophagostomum radiatum* was 2.56% in calf as compared in adult animal (3.27%), *Haemonchus placei*was2.56% in calf as compared to adult (0%) and *Bunostomum phlebotomum*was2.56% in calf as compared to adult (1.63%). In the present study sex-wise prevalence revealed that female cattle were highly infected with nematodes than male cattle. A study revealed by Chanei *et al*. (2012) reported that the higher prevalence of infection with gastrointestinal nematodes was (29.89%) in female than in male calf (25.25%). While Asif Raza *et al*. (2013) revealed that prevalence of *Haemonchus placei* was 0% in male cattle and 1.44% in female cattle. Ghanem *et al*. (2009) reported the prevalence of 34.3% and 27.1% in female and male cattle calves, respectively. Similarly Raza *et al*.(2010) recorded prevalence in large ruminants as 72.72% in female and 39.46% in male. In present study of month-wise prevalence of gastrointestinal nematodes in cattle, five nematodes were prevalent almost throughout the year and *Ostertagia* was predominant in cattle. There were two peaks of nematodes infection recorded during late winter and spring (February/April) and late summer (August/September) seasons that correlated with favorable climate (rainfall, humidity and temperature). This variation depends upon different epidemiological factors (Urquhart *et al*.1996). Al-Shaibani *et al*. (2008) reported the highest fecal egg counts in September, whereas the lowest fecal egg counts in February. While Ghanem *et al*. (2009) stated that the EPG% vary between dry season (May to October) and wet season (November to April) as it is much higher in wet season than in dry season. Rehman *et al*. (2009) recorded maximum prevalence from January to September, while minimum parasitism was observed from October to December. Tariq *et al*. (2010) reported higher prevalence in summer and lower in winter. It was noted that high temperature and high humidity during summer season played an important role in the spread of disease. Maximum numbers of larvae were found to be infective during summer months and are responsible for the occurrence of diseases also recorded in present study. The present therapeutic study showed that Ivermectin was more effective than Levamisole and Oxfendazole against cattle nematodes. The higher reduction of EPG (86-100%) was

recorded in cattle administered Ivermectin followed by Levamisole (68-98%) and Oxfendazole (66-96%). Kakar *et al* (2014) recorded the faecal egg count reduction test of sheep in Balochistan with oxfandazole, levamisole and ivermectin were 97%, 98% and 99% respectively, which indicated the susceptibility of these anthelmintics against gastrointestinal ssnematodes. Patten *et al*. (2011) stated that anthelmintics have a pivotal role in controlling the effects of parasites. Demeler *et al* (2009); Saurez *et al* (2010) and Yagoob *et al*. (2011) reported that number of antelmintics belonging to various groups are being used to treat the nematodiosis in large ruminants. Ijaz *et al*. (2007) and Gasbarre *et al*. (2009) recorded variable efficacies in a number of studies conducted abroad and local condition. Kaplan *et al* (2004) reports of anthelmintic resistance in nematodes of cattle have been less common, and the general belief is that resistance is not yet an important issue in this host and stated that this is a very insensitive means to monitor the development of resistance because cases only become apparent once resistance reaches very high levels in a population. Liu *et al*. (2009) reported that uncontrolled and excessive use of anthelmintics may lead to the development of resistance in parasite population. Demeler *et al*. (2009) and Gasbarre *et al*. (2009) studies have recommended that testing of anthelmintic efficacy should be performed more intensively due to existence of drug resistance. Coles *et al*. (2006) and Gasbarre *et al*. (2009) have also ported the resistance against benzimidazole. Gasbarre *et al*. (2009) and Kaplan *et al*. (2012) stated that resistance to more recently introduced anthelmintics for ruminants nematode is an emerging problem throughout the world. Demeler *et al*. (2009); Gasbarre *et al*.(2009) and Kaplan *et al*.(2012) reported the resistance against macrocyclic lactones group contain ivermectin, eprinomectin and moxidectin.

Conclusion: The gastrointestinal nematodes are prevalent in all age groups and either sex of cattle with peak during summer. The FECRT based diagnosis is more accurate. The Ivermectin products are more effective against cattle nematodes followed by Levamisole and Oxfendazole. The present study indicated that no anthelmintics resistance were found against gastrointestinal nematodes infection of cattle in Qilla Abdullah. However it is recommended that the proper anthelmintic dose with 5-6 months interval and rotation of anthelmintics for minimize the nematodes infection and enhance cattle productivity.

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