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Prevalence and Intensity of Ovine Gastrointestinal Nematodes in Balochistan, Pakistan

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Abstract.- An epidemiological study was conducted to evaluate the prevalence and intensity of gastrointestinal nematodes of sheep in slaughter house at Quetta, Balochistan, from April 2011 to March 2012. A total of 960 gastro-intestinal tract samples (80 per month) of sheep were examined during the study period. The overall prevalence of gastrointestinal nematode was 49.47% with the intensity of 516.63 in sheep. The higher monthly prevalence of gastrointestinal nematodes was observed during April (65%) followed by November (62.5%) and October (57.5%). Statistically there was significant differences ($P<0.05$) amongst months. The genus-wise intensity among gastrointestinal nematodes was presented higher by *Haemonchus* (213.05), followed by *Trichostrongylus* (137.05), *Trichuris* (90.1), *Ostertagia* (44.21) and *Nematodirus* (32.21). Breed-wise prevalence of gastrointestinal nematodes was higher in Balochi (50.20%) than the Harnai breed (48.75%). While, sex-wise prevalence of gastrointestinal nematodes was higher in female (56.87%) as compared to the male (42.08%). Age-wise prevalence of gastrointestinal nematodes in sheep was higher in the age group of < 1 year (60.83%) followed >2 years (43.33%) and 1-2 years (41.25%). Statistically there was no significant difference $P > 0.05$ between breeds, sex and age groups. Five genera of gastrointestinal nematode parasites were recovered during study period. Among these *Haemonchus* was the highest (71.36%) prevalent followed by *Trichostrongylus* (58.49%), *Trichuris* (28.84%), *Ostertagia* (17.26%) and *Nematodirus* (9.47%).

Keywords: Prevalence, gastrointestinal nematodes, sheep, *Haemonchus, Trichostrongylus, Trichuris, Ostertagia*.

INTRODUCTION

Parasitic diseases remain a main constraint to animal production systems across all agro ecological zones throughout the world. The productive and reproductive potential of domesticated livestock is adversely impaired by clinical and sub-clinical helminthes diseases. Among helminthes, gastrointestinal nematodes significantly affect the production of sheep due to reduction in appetite, loss of body condition, anemia, hypoproteinaemia, impaired digestive absorptive efficiency, other pathogenic complications and even death of animals (FAO, 1974; Barger, 1982; Steel and Symons, 1982; Holmes, 1986). Khan *et al*. (1988a) reported 100% sheep in upland Balochistan infected with internal parasites. Ahmed *et al*. (2005) recorded overall helminthiasis of 23.75% in slaughtered sheep at Quetta. Razzaq *et al*. (2002) recorded 93% gastrointestinal nematodes of sheep in Asghara valley district Ziarat. Several epidemiological factors influence the parasitic diseases in small ruminants. These include weather conditions, husbandry practices and the physiological status of the animals and for sustainable and normal control of gastrointestinal nematodes of sheep a comprehensive knowledge of epidemiology is a prerequisite (Pal and Qayyum, 1992; Wall *et al*., 2004; Keyyu *et al*., 2005). Therefore, the present study has been designed to assess the epidemiological factors contributing gastrointestinal nematodosis in sheep production system of Balochistan.

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MATERIALS AND METHODS

Experimental design

The present study was conducted from April 2011 to March 2012, during which a total of 960 gastrointestinal tracts (40/fortnightly) samples of sheep (Balochi and Harnai breed) from Quetta district abattoir was collected randomly and transferred to the Disease Investigation Laboratory of Livestock Department, Quetta for isolation and identification of gastrointestinal nematodes. A complete record of the animal *i.e.,* age, sex and breed were maintained at the time of sampling. Age of sheep was determined by the presence of incisors teeth in the lower jaw (Khan, 1969). Sampled animals were segregated into three age groups *i.e.*, < year, 1-2 year and > 2 year of age. Sheep breeds were identified based on their phenotypic characteristics (Hasnain, 1985).

Parasitological protocols

Nematodes from the abomasums, small and large intestine were isolated within four hours after the slaughtering of animals. Abomasums, small and large intestine were ligated at omasal-abomasal, abomasal-duodenal and ileo-caecal junctions to prevent worms spilling from one location to another. The abomasum was cut opened longitudinally, the contents of abomasum was poured in a 10 L bucket. Abomasal wall was washed thoroughly under a stream of water from a tap and mucous membrane rubbed with thumb finger to remove any adhering worms to it in the same bucket. The contents of bucket were sieved through a wire mesh screen with an aperture of 0.15 mm, and then washed with a stream of water. The screen having food materials and worms was inverted into another bucket and then washed with stream of water. More water was added to make up total volume of 4L which was then agitated vigorously and sample was taken by wide mouthed pipette. The sample was transferred to a measuring cylinder until a total volume of 40 ml. Small quantity of this 40 ml were placed in a Petri dish having parallel line and examined under stereomicroscope for worms count. The large and small intestine were processed same as abomasam. Worms from abomasam, large and small intestine were collected and counted (Charles and Baker, 1988). The collected nematodes were washed in physiological saline (0.89 gm/100 ml distilled water) and were fixed in 70% alcohol for 24 hours. The nematodes were then transferred to a vial containing a hot mixture of 70% ethyl alcohol (Merck) and glycerol (Merck) equal parts. The worms were kept in this vial partly covered until all ethyl alcohol was evaporated and worms were left in pure glycerol. The worms were cleared in lacto phenol and then placed on microscopic slide and mounted in pure glycerol. Excess of glycerol was removed with the help of filter paper and the edges of the cover slip were sealed with slide sealer. The gastrointestinal nematodes were examined under 4x and 10x magnification of compound microscope and identified using standard keys and morphological characteristics described by (Yamaguti, 1961; Maff, 1979; Soulsby, 1982; Urquhart *et al*., 1996).

Statistical methods

The mean intensity was calculated (total number of worms recovered/ number of infected sheep) and the prevalence of infection (number of infected host/number of surveyed sheep). The intensity for each genus (number of worms/infected sheep) and the frequency (number of worms of one particular genus/total number of worms recovered) were calculated. The data collected from this study were analyzed by using Graph Pad Prism-5 computer statistical package. The prevalence of different nematodes in different age, breeds and sex-wise were analyzed through one way analysis of variance at ($P<0.05$). Metrological data were calculated on simple mean and percentage basis.

RESULTS

The overall prevalence of gastrointestinal nematodes was 49.47% with intensity of 516.63 in sheep at Slaughter-house Quetta, Balochistan. The higher monthly prevalence of gastrointestinal nematodes was observed during April (65%) followed by November (62.5%) and October (57.5%). Similarly, higher monthly mean intensity was recorded during October (65.68) followed by November (54.94). Statistically there was significant difference $P < 0.05$ among months (Table I).

Table I.- Month, age, breed and sex-wise gastro-intestinal nematodes prevalence, intensity and frequency in slaughtered sheep at Quetta.

Parameter	Months	Prevalence	Intensity	Frequency
Months	April, 2011	65	46.73	
	May, 2011	57.5	42.52	
	June, 2011	48.75	40	
	July, 2011	42.5	33.05	
	August, 2011	52.5	46.73	
	September, 2011	50	46.31	
	October, 2011	57.5	65.68	
	November, 2011	62.5	54.94	
	December, 2011	47.5	41.47	
	January, 2012	41.25	37.47	
	February, 2012	36.25	28.42	
	March, 2012	32.5	33.26	
Genus	*Haemonchus*	71.36	213.05	0.41
	Ostertagia	17.26	44.21	0.08
	Nematodirus	9.47	32.21	0.06
	Trichostrongylus	58.94	131.05	0.26
	Trichuris	28.84	90.1	0.17
Breed	Balochi	50.20		
	Harnai	48.75		
Sex	Male	42.08		
	Female	56.87		
Age	Under 12 months	60.83		
	1-2 Year	41.25		
	> 2 Year	43.33		
Overall		40.47		

Meteorological record of district Quetta

The meteorological data of district Quetta from April 2011 to March 2012 were obtained from Land and Water Resources Research Program, Arid Zone Research Centre (PARC), Quetta. The rainfall was very low (less than 20 mm) through out the study period, however higher rainfall recorded during April 2011 and January to March 2012. The temperature was higher (above 30°C) during June to August 2011, while lower during the rest of study period. Similarly, humidity was also lower (30%) round the year except January to March 2012 (Fig.1).

Genus-wise intensity and frequency of nematodes

In the present study, GIT samples (n=960) assessments showed intensity of five nematodes genera in sheep at Quetta slaughter-house. Among these higher intensity of *Haemonchus* (213.05) was recorded followed by *Trichostrongylus* (137.05), *Trichuris* (90.1), *Ostertagia* (44.21), *Nematodirus* (32.21) during the one year study. Similarly, the higher frequency was also presented by *Haemonchus* (0.41) followed by *Trichostrongylus* (0.26), *Trichuris* (0.17), *Ostertagia* (0.08) and *Nematodirus* (0.06) in sheep (Table I).

Breed-wise prevalence of nematodes

In present study, GIT samples of two sheep breeds *i.e.*, Balochi and Harnai (n=480 from each breed) were analyzed for presence of nematodes. The results showed that overall prevalence of gastrointestinal nematodes was 49.47% (475/960). The Balochi breed showed higher (50.20%)

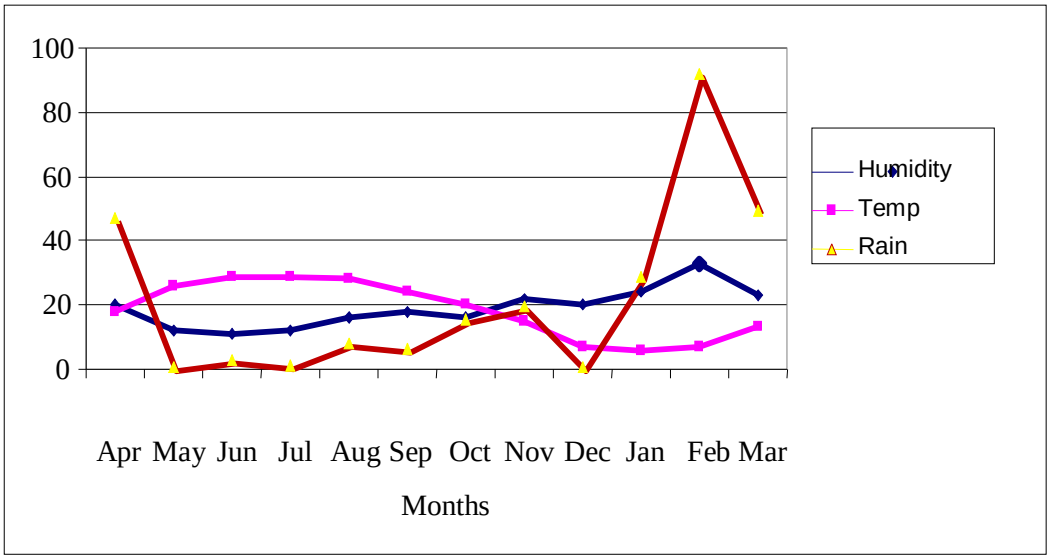

Fig. 1. Meteorological record of district Quetta during April 2011 to March 2012.

prevalence of gastrointestinal nematodes than Harnai breed (48.75%) (Table I). Statistically there was no significant difference P>0.05 between breeds.

Sex-wise prevalence of nematodes

The prevalence of gastrointestinal nematodes was higher in female (56.87%) as compared to male (42.08%), while the overall prevalence was 49.47% (Table I). Statistically there was no significant difference P > 0.05 between either sex of sheep.

Age-wise prevalence of nematode

The present study results showed that the sheep under < 1 year age showed higher prevalence (60.83%) of gastrointestinal nematodes followed by > 2 years (43.33%) and 1-2 years (41.25%) age groups (Table I). Statistically there was no significant difference among age-wise at P > 0.05.

Genus-wise prevalence of nematode

In present study, five genera of gastrointestinal nematode parasites were recovered in slaughtered sheep. Among these *Haemonchus* was found highest (71.36%) prevalent followed by *Trichostrongylus* (58.49%), *Trichuris* (28.84%), *Ostertagia* (17.26%) and *Nematodirus* (9.47%) (Table I).

DISCUSSION

The overall prevalence of gastrointestinal nematodes as 49.47% with intensity of 516.63. These findings are in agreement with Shimelis *et al*. (2011) who observed the overall prevalence of gastrointestinal nematodes as 47.67% in North Gondar zone of Northwest Ethiopia. Khan *et al*. (2010) reported nematodes prevalence (44.17%) which is almost close to the present study. However, Lateef *et al*. (2005) reported higher prevalence (65%) of gastrointestinal nematode than the present study. Abunna *et al*. (2009) reported 83.6% and even higher (91.7%) by Sing *et al*. (1997) at India and 91.32% by Tefera *et al*. (2011) in South-Western Ethiopia, respectively. The reasons might be due to variation in environmental conditions such as the related results of present study with Ethiopia due to similar dry environment, while higher prevalence in different areas like Punjab, India due to hot and humid environment.

The month-wise higher prevalence of gastrointestinal nematodes was observed during April, November and October in the present study which was in agreement with Nginyi *et al*. (2001). They recorded higher monthly prevalence of gastrointestinal nematodes during the months of April and October which might be due to the

similarities in the climatic conditions. Githigia *et al*. (2001) also reported two peaks of nematodes prevalence. The first peak was during August/September which may be due to uptake of larvae during June/July and the second peak was during November/December (due to uptake of larvae during the short rainy season). Similarly in the present study two peaks were recorded during April and November which might be due to favorable environmental conditions as described by Rafique *et al*. (1997) that parasitic burden of range-animals in an area might be associated with different climatic conditions, husbandry practices and other managemental factors. Qamar (2009) described the role of meteorological data like humidity, rainfall and temperature which was correlated with the occurrence of the disease. Difference in the incidence of different gastrointestinal nematode parasites in the present study may be due to differences in arid environment and nematodes susceptibility as mention by Durrani *et al*. (1981) that incidence of few species of gastrointestinal nematode parasites was relatively higher and some other species had relatively lower incidence under arid conditions of upland Baluchistan as compared to semi-humid, subtropical Punjab province.

In present study the prevalence of gastrointestinal nematodes in Balochi breed was higher (50.20%) than Harnai breed (48.75%). These findings were in agreement with Mushtaq and Tasawar (2011) who observed higher infection of gastrointestinal nematodes in Kacchi breed than Lohi breed. Tasawar *et al*. (2011) also observed higher infection of gastrointestinal nematode parasites in Lohi breed (87.09%) than Awassi (76.9%) and Hisardale breed (74.2%). Chaudhry *et al*. (2009) also pointed out that the *H. contortus* was the most ubiquitous and predominated gastrointestinal parasite in Bulkhi ewes followed by *Trichostrongylus* spp. and *Oesophagostomum columbianum*. AL-Shaibani *et al*. (2008) recorded Kooka breed slightly more susceptible as compared to Kacchi and Dumbi breeds. In contrast to the current results, Chaudry *et al*. (2007) recorded significant variation in larval counts among sheep breeds from different ecologies, while no difference was recorded among different breeds at same ecology. Similarly, Abunna et al. (2009) observed significant difference between host risk factors in prevalence of different nematode species. Urquhart *et al*. (1996) stated that the susceptibility by various breeds of animal to parasites varies and is genetically determined.

In present study the prevalence of gastrointestinal nematodes was higher in female (56.87%) as compared to male (42.08%). These findings were also in agreement with some researchers (Maqsood *et al*., 1996; Lateef *et al*., 2005; Al-Shaibani *et al*., 2008; Shimelis *et al*., 2011; Khan *et al*., 2010) who observed relatively higher prevalence of gastrointestinal nematode in female animals as compared to male. This may be due to the gestation stress, pre- parturient parasitic rise as the pre-disposing factors for higher prevalence in female stock. In contrast to present study Mushtaq and Tasawar (2011) observed the prevalence of gastrointestinal nematode parasites was higher in male sheep (81.4%) as compared to female sheep (73.1%). Barger (1993), Bilbo and Nelson (2001) reported that such differential prevalence of gastrointestinal nematodes in sheep may be due to stimulatory effects of estrogens and inhibitory effect of androgens on immune responses. While, Qamar (2009) was of view, that the animals of either sex were equally affected by the helminths. Similarly, Urquhart *et al*. (1996) correlated the hormonal difference in either sex with parasitic susceptibility and found that males are 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. In contrast, Raza *et al*. (2009) clarified that the higher prevalence in females might be due to lower resistance on the part of their reproductive events and insufficient/ unbalanced feed against higher need. The female animals generally harbored a significantly higher worm burden than male animals due to the enhanced grazing of females during lactation and their low resistance during pregnancy and parturition. Silva *et al*. (2011) also supported the rearing systems and observed that the goats in the organic system had higher fecal egg counts ($P<0.05$) than the goats in the conventional system.

Age-wise results in the present study

presented higher prevalence of gastrointestinal nematode parasites in sheep of less than one year age (60.83%) followed by >2 years (43.33%) and 1-2 years (41.25%) age groups. These findings were also in line with other researchers (Lateef *et al*., 2005; Qamar, 2009; AL-Shaibani *et al*., 2008; Khan *et al*., 2010; Shimelis *et al*., 2011) who recorded higher prevalence of gastrointestinal nematodes in sheep of less than one year <1 (69.2%), followed by 1–2 years (50.8%) and >2 years (37%) old animals. In contrast to the present study, Tasawar (2011) reported higher prevalence of gastrointestinal nematodes in older age animals than younger. This might be due to low immunity in younger than the older ones. Urquhart *et al*. (1996) also stated that a significant immunity develops with age against a few parasites in adult stock. Radostits *et al*. (1994) pointed out that two age groups of sheep most commonly affected are weaned lambs and yearlings. They added that the sheep over 18 month of age are less commonly affected because of immunity, resulting from previous infection. The low nematode prevalence in younger sheep at might be due to grazing on (nearby home) low contaminated pastures and supplemental feeding (barley grain, green wheat, barseem etc.). While higher nematode prevalence in adult sheep might be due to grazing on larger area of pastures being contaminated with various flocks and different stress conditions like climate, long daily traveling and gestation etc. In contrast, Abunna *et al*. (2009) stated that there was no significant difference of sheep nematodes prevalence between age and month. Young animals also get infected with internal parasitic ova from the contaminated pastures being spread for the female animals during gestation period because of higher parasitic load at this stage.

Five genera of gastrointestinal nematode parasites were recovered from gastrointestinal tract during study period. Among these *Haemonchus* was highest (71.36%) in prevalence followed by *Trichostrongylus* (58.49%), *Trichuris* (28.84%), *Ostertagia* (17.26%) and *Nematodirus* (9.47%), respectively. These findings are in agreement with Lateef *et al*. (2005) who recorded the highest prevalence of *H. contortus* (61.5%) followed by *Trichostrongylus* spp. (46.1%) and *Ostertagia* spp (33.0%). Similarly, Asif *et al*. (2008) reported that *Haemonchus* was higher in prevalence (80.64%) followed by *Trichuris* (32.25%) and *Nematodirus* (29.03%), respectively. Gadahi *et al*. (2009) recorded the prevalence of *Haemonchus* (28.88%), *Trichuris* (40%) and *Nematodirus* (11.11%) in sheep. Nginyi *et al*. (2001) recorded the genera of nematodes *Haemonchus* and *Trichostrongylus* as 33% and 29%, respectively. Abunna *et al*. (2009) observed the prevalence of *Haemonchus* (78.1%) and *Trichostrongylus* spp. (90.4%) in sheep. Zeryehun (2012) recorded genera-wise prevalence in sheep for strongly type eggs, *Strongyloides* sp., *Trichuris* sp., as 39.84%, 17.45% and 7.81%, respectively. Kantzoura *et al*. (2012) reported prevalence in sheep for *Nematodirus* spp. as 1.1% and for *Trichuris* spp. as 2.9%. Mushtaq and Tasawar (2011) recorded prevalence of *Haemonchus contortus* and *Trichuris*, 6.50% and 5.73%, respectively. While Al- Shaibani *et al*. (2008) recorded higher prevalence of *H. contortus* (24.6%) which was found to be predominant of gastrointestinal nematode parasites while *Trichostrongylus* spp. (18.0%) was the next most prevalent specie. while Khan *et al*. (1988b) reported the same parasites in upland districts of Baluchistan. Difference in the occurrence of different gastrointestinal nematode parasites in the present and other studies carried out in different locations might be due to different ecologies, temperature and pastures. Durrani *et al*. (1981) reported that occurrence of gastrointestinal nematodes was higher and lower in arid conditions of upland Baluchistan as compared to semi-humid, subtropical climate of Punjab province. Radostits *et al*. (1994) pointed out that warm, wet weather provides favorable conditions for the translation of eggs to larvae in the majority of helminthes. The areas having severe summer and dry winter reduced the parasitic burden on the local livestock. It was observed in present study that most of the flocks were sedentary and they were under strict confinement which leads to high risk of helminthes infection (Anene *et al*., 1994). In present study among five genera of gastrointestinal nematode *Haemonchus* was most prevalent nematode. It might be due to its biotic potential which justified the percentage of infection (Nginyi *et al*., 2001). The *H. contortus* larval development occurs when there is high temperature,

rainfall and high humidity favorable microclimate of herbage and faeces (Urquhart *et al*., 1996). *Trichostrongylus* was next most prevalent nematode parasite in the present study. These findings are in contrast to those of Suarez and Busetti (1995) mentioned that *Trichostrongylus* populations were high in autumn and reached their peaks in June to July, while the highest larval availability was in autumn. *Trichostrongylus* species has capability to developed and survive at lower temperature (Mallet and Kerboeuf, 1986). Furthermore, *Trichostrongylus* is the dominant parasite in temperate areas with the cooler environment creating optimum condition for larval development (O'Connor *et al*., 2006) the overall prevalence of *Ostertagia* spp. was 17.26% in the present study. Some researchers (Tasawar *et al*., 2011; Umur and Yukari, 2005; Craig *et al*., 2006) recorded higher prevalence of *Ostertagia* spp. that were 77%, 64.6%, 73.58%, 94.8%, 80%, 75%, respectively and Sajid *et al*. (1999) recorded lower (16.2%). These differences in the percentage reported by different researchers could be due to different managemental practices (Lindquist *et al*., 2001), natural resistance based on genetic background and anthelmintic treatment (Soulsby, 2005) and geo-climatic factors of the regions (Pal and Qayyum, 1993). In present study the prevalence of *Trichuris* was 28.84%. These findings are near to Asif *et al*. (2008) who recorded 32.25% prevalence of *Trichuris*. Gadahi *et al*. (2008) recorded higher than these (40%) while others (Reda *et al*., 2011; Zeryehun, 2012; Mushtaq and Tasawar, 2011) reported the lower prevalence *i.e*. 5.8%, 2.9% and 5.73%, respectively. These differences in the percentage reported by different researchers might be due to differential management practices and geo- climatic factors of the regions (Pal and Qayyum, 1993). The prevalence of *Nematodirus* was 9.47% in present study. These findings are close to the findings of Asif *et al*. (2008) who reported prevalence in sheep for *Nematodirus* spp. as 11.11% and Kantzoura *et al*. (2012) reported lower prevalence 1.1%. Some particular parasites, *Nematodirus*, have no obvious seasonal pattern of occurrence and chill temperature requirement for larval development are important factors for their prevalence during different period of year. Similarly, some climatic factors like warmness and moisture favor development and allow the accumulation of large numbers of infective stages on the rangelands (Urquhart *et al*., 1996).

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