

## Presence of Strongyle Infection in Thoroughbred Horses of Punjab Province, Pakistan

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<https://doi.org/10.12982/CMUJNS.2020.0015>

Received: January 6, 2019

Revised: April 18, 2019

Accepted: July 15, 2019

### ABSTRACT

*Strongyle infection among horses is complex and produces an inflammatory enteropathy resulting in impaired intestinal motility and microcirculation. In this study, presence of strongylosis was determined by fecal examination for nematode eggs in thoroughbred horses of Punjab Province, Pakistan. A cross sectional study was conducted from January to December 2017 in four districts of Punjab province namely Sahiwal, Lahore, Sargodha and Mandi Baha-ud-Din. A total of 2,400 fecal samples were collected from horses and examined by direct smear and floatation method. Presence was calculated according to region, month, season, age and gender. In addition, the effect of meteorological factors such as temperature, humidity and rainfall were also analyzed. The findings revealed that out of 2,400 samples, 973 (40.54%) were found positive for strongylosis. There was a statistically significant difference between seasons and months with disease occurrence. The parasite and host form an association to form an epidemiological danger. The existence and localization of this association must be recognized well in time to control the problem.*

**Keywords:** Strongylosis, Nematodes, Horses, Presence, Infestation

### INTRODUCTION

Livestock, an indispensable part of agriculture sector, epitomizes a foremost national resource. It plays a vital role in attaining and improving living standards of both developing and developed nations (Smith et al., 2013). According to Pakistan Livestock Census, equine population is 4.76 million

approximately. The livestock backs about 56.3% of the value of agriculture and virtually 11% to the agricultural gross domestic product in the country (Rehman et al., 2017). Many factors influence the health and productivity of equines. Parasitism is an imperative limitation of efficacious horse rearing around the world and many species of parasites can cause serious infections in equines especially horses (Uslu and Guclu, 2007). Parasitic ailments can be a major constraint for the economy of poor nations and are seldom linked to high mortality rates and have inimical effects on the stakeholders of food security (Goraya et al., 2013). They are accountable for immunosuppression, predisposing the animals to other illnesses. Some parasitic infections may even cause deaths when the control measures remain unkempt (Fleurance et al., 2007). Strongyle nematodes are the chief internal parasites of the equines covering almost 50 % of the over 100 species and documented with an infection rate of 58.5% (Nielsen, 2012). Henceforth, the detection of gastrointestinal nematode infections holds an important position in studying parasite life-cycles, epidemiology, population biology and systematics, essential for the diagnosis and control.

It is therefore mandatory to investigate the disease dynamics for better prevention and therapeutic strategies. Research reports on horse parasites in Pakistan are extremely scanty with almost non-existent scientific reports on the presence of strongylosis in horses and their therapies. This study focused on the documentation of presence and intensity of strongylosis in thoroughbred horses in four districts of Punjab province to procure baseline data so as to design effective control options.

## MATERIALS AND METHODS

### Study area and sample collection

The study was undertaken from January to December 2017 in 4 selected districts (Lahore, Sahiwal, Sargodha and Mandi Baha-ud-Din) of Punjab, Pakistan. A total of 2,400 faecal samples of thoroughbred horses (1,200 geld & 1,200 mares), 600 per district (50 per month per district) for a period of 1 year were examined randomly for the presence of strongyle eggs. Simple random sampling method was used to select individual study animals.

In Sahiwal, samples were collected from remount stallion stables of Horse Mules and Cattle Breeding of Qadirabad, Yousafwala, Iqbalnagar, Kassowal, Noorshah, Harappa and Ghaziabad towns. In these areas the Government of Pakistan (Ministry of Defence) has leased out lands to the tenants for the breeding and rearing of equines. These areas comprise a nursery for supply of horses to Pakistan Army for operational use in the hilly terrain.

Lahore district is hub of equisterian activities where polo tournaments and horse races are routine features. The survey was carried out at Military Veterinary Detachment Lahore Cantonment, Lahore Garrison Saddle & Polo Club Fortress

Stadium, Lahore Polo Club, Lahore Race Club and from Manga Mandi, Sunder, Kala shah Kaku, Bedian, Barki and Shahdra towns.

The Remount Depot Sargodha and Shapur, Mitha Lak, Chak 71 NB, Khan Muhammad Wala, Jhal Chakian, Shaheen Abad, Kot Sultan, Military stud farms were visited for sample collection in Sargodha. The equines reared in these towns are well known for their excellence in polo and tent pegging. The population of equines is maximum in this district compared with other districts of Punjab province.

In Mandi Baha ud din the samples were collected from North Charge / Horse breeding stud of Remount Depot Mona.

Information regarding the animal was collected according to a pre-designed questionnaire. Systematic investigation was done according to the parasitic infection. Clinical examination of each animal was also performed; rectal temperature, pulse rate, respiration rate, palpation of lymph nodes, colour of conjunctiva, texture of faeces, urine colour, presence of any swelling on the extremities and presence of any ectoparasite on the body was documented.

**Study design and methodology**

The villages and horse stud farms of four districts mentioned above were visited every month for the entire course of study. Simple random sampling method was used to select individual study animals. On each visit, a total of 200 faecal samples were collected (50 from each district per month). These samples were examined by direct smear and flotation techniques. Presence of infection was recorded as month, season and area wise. The age, sex and breed of each animal was also furnished. The presence of disease in different districts of Punjab province was recorded following the formula described by Neilson et al. (2014).

$$Presence = \frac{No. \text{ of horses infected with strongyles}}{No. \text{ of horses examined}} \times 100$$

To record the seasonal presence, year was apportioned into four seasons i.e. winter (Nov-Feb), spring (Mar-Apr), summer (May-Aug) and autumn (Sep-Oct). To record the age wise presence, the horses were divided in four age groups (1-5, 6-10, 11-15 and 16-20 years). The presence percentage of an age group was calculated out of total number of horses in that particular age group. The age detail of all the 2,400 examined horses has been shown in Table 1.

**Table 1.** Age structure of examined horses.

Age (years)	Sahiwal	Lahore	Sargodha	Mandi Bah-ud-Din	Total
1-5	190	193	188	184	755
6-10	213	230	251	256	950
11-15	124	140	120	130	514
16-20	73	37	41	30	181
Total	600	600	600	600	2,400

Presence in relation to meteorological factors including temperature, humidity and rain fall was also determined. Meteorological data for Sahiwal, Lahore, Sargodha and Mandi Baha-ud-Din districts was collected from Pakistan Meteorological Department Gulberg III, Lahore (Table 2).

**Table 2.** Meteorological Data of Sahiwal, Lahore, Sargodha and Mandi Baha-ud-Din.

District	Factors		Jan	Feb	Mar	Apr	May	June	July	Aug	Sep	Oct	Nov	Dec
Sahiwal	Avg. temperature (°C)	Min	3.8	6.6	12.3	17.3	22.1	26.3	26.4	25.9	22.8	17.9	10.5	6.6
		Max	20.1	20.9	26.3	33.9	37.9	41.8	38.6	35.3	35.1	32.6	26.7	21.4
		Mean	11.9	13.8	19.3	25.6	29.0	34.5	32.5	30.6	28.9	25.3	18.6	14
	Avg. Relative Humidity (%)	Min	53	55	51	35	33	34	49	51	50	48	47	57
		Max	90	88	83	68	58	55	71	74	80	84	86	88
		Mean	71.5	71.5	67	51.5	45.5	44.5	60	62.5	65	66	66.5	72.5
	Rainfall (mm)			0.1	25.2	58.2	42.5	16.3	15.2	76.1	60.1	34.1	1	4
Lahore	Avg. temperature (°C)	Min	4.4	6.9	12.5	17.8	22.8	27.8	26.8	26.5	23.9	19.4	12.2	6.1
		Max	18.8	20	25.6	32.9	37.7	42	36.7	36.5	33.6	31.7	26.3	20
		Mean	11.6	13.5	19.0	25.4	30.5	34.9	31.8	31.5	28.8	25.6	19.3	13.1
	Avg. Relative Humidity (%)	Min	59	58	52	36	32	32	56	54	64	51	47	61
		Max	87	85	75	60	57	49	71	72	83	75	80	86
		Mean	73	71.5	63.5	48	44.5	40.5	63.5	63	73.5	63	63.5	73.5
	Rainfall (mm)			6.1	25.9	49.5	115.5	33.8	44	41.1	27	31.1	0	5.2
Sargodha	Avg. temperature (°C)	Min	4.6	7.2	13.3	17.8	22.9	28.1	28	26.4	24.4	19.0	10.3	6.7
		Max	20.2	20.3	24.5	31.6	36.3	41.9	37.4	36.1	33.8	30.8	26.4	20.4
		Mean	12.4	13.8	18.9	25.2	29.6	35	32.7	31.3	29.1	24.9	18.4	13.6
	Avg. Relative Humidity (%)	Min	55	58	53	48	43	55	56	60	59	57	55	62
		Max	89	88	80	72	61	73	78	81	81	83	85	88
		Mean	72	73	66.5	60	52	64	67	70.5	70	70	75	75
	Rainfall (mm)			22.2	22	35.2	34	39.4	39	76.1	96	135.1	14.3	4
Mandi Baha-ud-Din	Avg. temperature (°C)	Min	5.6	8.2	12.9	17.5	22.1	26.5	26.7	25.8	23.7	19.2	11.2	7.4
		Max	20.2	20.5	25.3	31.7	36.4	41.9	36.6	35.9	33.3	31.4	25.5	19.6
		Mean	12.9	14.4	19.1	25.1	29.3	34.2	31.7	30.9	28.5	25.3	18.4	13.5
	Avg. Relative Humidity (%)	Min	68	63	58	46	48	32	62	64	67	62	60	67
		Max	80	90	85	73	78	53	82	84	87	88	91	92
		Mean	74	76.5	71.5	59.5	63	43.5	72	74	73.5	75	75.5	79.5
	Rainfall (mm)			1.3	20.3	126.3	58.5	40.2	41.6	178	189	235.1	25.3	18

Note: \*Source = Pakistan Meteorological Department.

### Parasitological examination

Approximately five to ten grams of fresh faeces were collected directly from the rectum in separate polythene bags, properly labelled and transported to the laboratory of Department of Zoology, University of the Punjab, Lahore, Pakistan. They were subjected to direct microscopic examination on the same day to detect the worms and eggs (Zajac and Conboy, 2012). Three slides were examined from each sample and an average was taken. The samples found negative were re-examined by using centrifugal floatation method before declaring as negative.

The positive samples were further subjected to eggs per gram (EPG) counting to find out the number of eggs per gram of the faeces by the method described by Mohammed et al. (2015). As per the EPG count, the infection was rated as light (50-799 EPG), moderate (800-1,200 EPG) and severe (>1,200 EPG) (Haimanot et al., 2015).

## Statistical analysis

To analyze and compare the mean  $\pm$  SEM values for epidemiology, the statistical analysis was performed by using analysis of variance (ANOVA) at  $P = 0.05$ . Chi square test was performed to assess the dependence of degree of infection on certain risk factors. The data were analyzed by using GraphPad Prism programme version 5.00.

## RESULTS

### Overall presence in Punjab Province

A total of 2,400 thoroughbred horses were examined of which 973 were found positive for strongylosis. The overall presence was thus 40.54%.

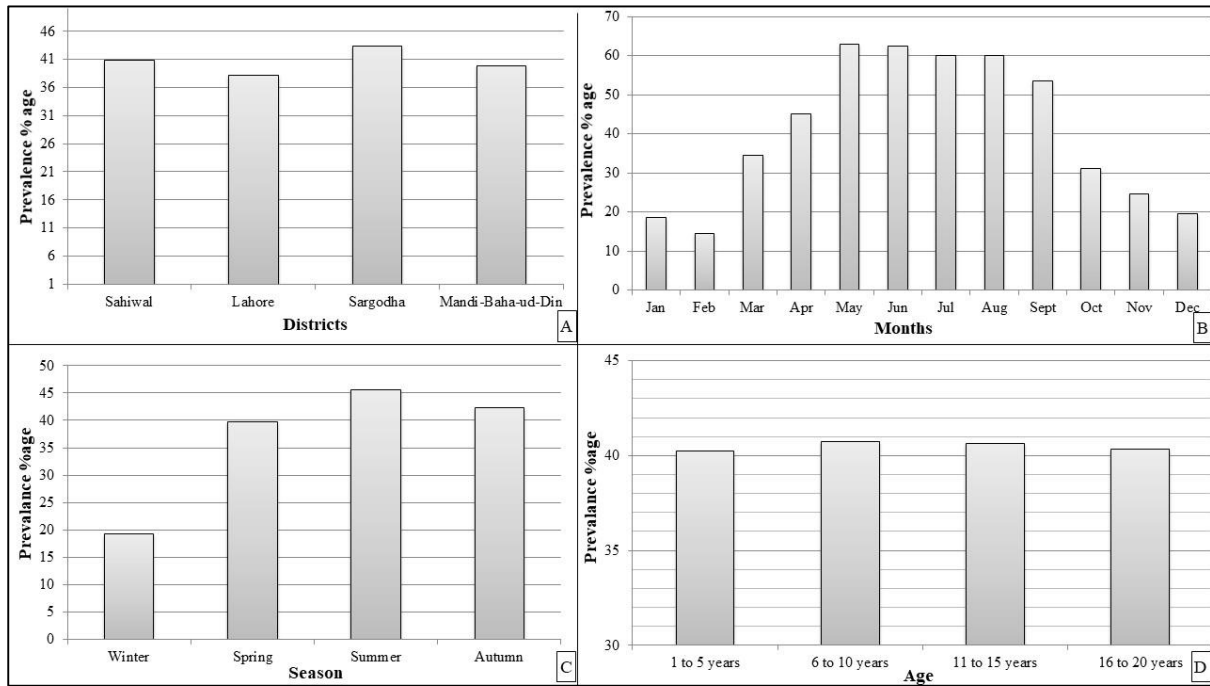
The strongylosis in horses in all the four districts of Punjab indicated that infection rate was highest at Sargodha district (43.33%) followed by Sahiwal (40.83%) then Mandi Baha-ud-Din district (39.83%) whereas the lowest was noted at Lahore district (38.16%) (Figure 1A). Details are shown in Figure 1A. There was no statistically significant difference ( $P = 0.05$ ) in presence of strongylosis infection of horses between four sites.

Analysis of strongylosis presence of horses by months showed that there were statistically significant month-wise variations ( $P < 0.05$ ). The higher infection presence (63%) was noted during the month of May followed by June while the lowest (12%) was noted during February (Figure 1B).

Season wise analysis of the presence rate revealed the maximum frequency (61.37%) of infection documented during summer followed by autumn then spring whereas the lowest (19.25%) was recorded during winter. The data suggest a highly significant increase in presence percentage during summer season at  $P = 0.05$  (Figure 1C).

Percentage of animals infected did not appear to differ with the age of horses (Figure 1D). The results indicated almost equal presence of disease in all the age groups (40.26%, 40.73%, 40.66% and 40.33% for horses aged between 1-5, 6-10, 11-15 and 16-20 years respectively) with no significant difference at  $P = 0.5$ .

Gender based analysis indicated the presence to be 40.91% in geldings followed by 40.16% in mares. The findings suggested that there was an insignificant relationship between the gender and disease frequency at  $P = 0.05$ . It can be inferred that incidence of the disease was independent of animal age.



**Figure 1.** Presence percentage of strongylosis in horses (A) District-wise presence (B) Month-wise presence (C) Season-wise Presence (D) Age-wise presence.

**Infection presence with respect to the environmental factors**

The maximum/minimum and monthly presence of strongylosis with relation to environmental temperature, mean humidity and rainfall at four districts of Punjab has been shown in Table 3. It was noted that the infection was directly proportional to the environmental temperature which had a statistically significant effect ( $P < 0.05$ ). The maximum presence percentage was observed between the temperature range of 28.9 to 32.7 °C whereas minimum values were documented between 13.0 to 14.3 °C in all the four districts. Sargodha district harbored the highest number of infected individuals (76%) at mean temperature of 32.7 °C and least number of cases (14%) were reported at 13.5 °C. In case of Mandi Baha-ud-Din, the presence of strongylosis was recorded maximum (68%) at 29.2 °C and minimum at 13.5 °C. The strongylosis burden for Sahiwal was documented maximum (66 %) at mean temperature 28.9 °C and minimum (10%) at 13.7 °C. Overall presence of strongylosis at Punjab province was recorded maximum (67 %) at mean temperature of 31.2 °C and minimum presence (12.5%) at mean temperature of 13.6 °C.

The effect of relative humidity on the presence of strongylosis infection indicated that moderate humid environment favors the presence of strongylosis among horses. The relationship between the relative humidity and infection was statistically significant at  $P = 0.05$ . As the level of humidity escalated, progressive decrease in the incidence of the disease was witnessed. Maximum presence was recorded between 63.5% to 65% mean relative humidity, however the disease

frequency dejected above mean relative humidity of 70% at all the areas studied. Overall Presence of strongylosis at Punjab province was recorded maximum (67%) at mean relative humidity of 64.62% and minimum presence (12.5%) at mean relative humidity of 73.37%.

The statistical analysis of the effect of rainfall on infection presence did not reveal the significant results at  $P = 0.05$ . The presence of infection did not alone support the growth of parasite in the horses. Presence of strongylosis at Sahiwal district was recorded maximum (66%) at 34.1 mm rain fall, Lahore (58%) at 41.1 mm rain fall, Sargodha (68%) at 76.1 mm rain fall and Mandi Baha-ud-Din (68%) at 79.2 mm rain fall. Presence of strongylosis at Sahiwal district was recorded minimum (10%) at 25.5 mm rain fall, Lahore (14%) at 11 mm rain fall, Sargodha (14%) at 22.2 mm rain fall and at Mandi Baha-ud-Din (12%) at 20.3 mm rain fall. Overall Presence of strongylosis at Punjab province was recorded maximum (67%) at 38.6 mm rain fall and minimum presence (12.5%) at 19.62 mm rain fall.

### **Degree of strongylosis infection with different risk factors**

The samples declared positive through qualitative parasitological examination were subjected to EPG count to estimate the degree of infection. Accordingly, out of 973(40.54%) positive specimens, 383(39.3%), 334(34.2%) and 256(26.3%) were found to be lightly, moderately and massively infested respectively. Furthermore, the degree of infection was assessed with respect to gender, age group and season and the results are summarized in Table 4. The statistical analysis (Chi-square test) of the data revealed that the severity of infection was influenced by all the risk factors under consideration ( $P = 0.05$ ).

## **DISCUSSION**

The presence of strongyles in the study area may be attributed to the suitability of the climatic conditions (Table 2) of all the four districts for survival and transmission of the parasites. The present study when compared to different study areas showed alike infection rate of strongylosis nematode. Pilo et al. (2012), during a study on 46 Sardinian horses suffering from strongylosis in Italy demonstrated that *S. vulgaris* larvae were found in 39 % whereas the detection rate in faeces was only 4%. Saeed et al. (2010) reported that infection rate of strongyles (*S.vulgaris*, *S.edentatus* and *S.equi*) was 58.5 % among the equines studied.

The overall month-wise presence percentage ranged from 14.5 to 62.5% in a calendar year. As regard to season wise presence, it was the highest (61.37%) during summer followed by autumn then spring and the lowest (19.25 %) during winter. The present findings are in good agreement with that of

Jubb et al. (1985), Krecek et al. (1987) and Saeed et al. (2010) wherein higher eggs excretion was observed in spring and summer. This may be because that elevated temperatures are apt for larval development and colossal contamination of infective larvae may take place in summers and early autumn when young susceptible horses are present (Wannas et al., 2012).

**Table 3.** Presence of strongylosis with respect to environmental factors.

District	Factors	Jan	Feb	Mar	Apr	May	June	July	Aug	Sep	Oct	Nov	Dec	F value
Sahiwal	Avg. temperature (°C)	11.9	13.8	19.3	25.6	29.0	34.5	32.5	30.6	28.9	25.3	18.6	14	9.7432*
	Avg. Relative Humidity (%)	71.5	71.5	67	51.5	45.5	44.5	60	62.5	65	66	66.5	72.5	13.460*
	Rainfall (mm)	0.1	25.2	58.2	42.5	16.3	15.2	76.1	60.1	34.1	1	4	0	3.04773
	Presence of infection (%)	24	10	24	40	52	56	58	58	66	36	40	26	
Lahore	Avg. temperature (°C)	11.6	13.5	19.0	25.4	30.5	34.9	31.8	31.5	28.8	25.6	19.3	13.1	6.7544*
	Avg. Relative Humidity (%)	73	71.5	63.5	48	44.5	40.5	63.5	63	73.5	63	63.5	73.5	12.061*
	Rainfall (mm)	6.1	25.9	49.5	115.5	33.8	44	41.1	27	31.1	0	5.2	11	3.04773
	Presence of infection (%)	22	16	30	38	58	56	58	56	54	34	22	14	
Sargodha	Avg. temperature (°C)	12.4	13.8	18.9	25.2	29.6	35	32.7	31.3	29.1	24.9	18.4	13.6	7.2696*
	Avg. Relative Humidity (%)	72	73	66.5	60	52	64	67	70.5	70	70	75	75	11.802*
	Rainfall (mm)	22.2	22	35.2	34	39.4	39	76.1	96	135.1	14.3	4	1	0.00011
	Presence of infection (%)	14	20	42	50	74	76	68	62	54	28	18	14	
Mandi Bahauddin	Avg. temperature (°C)	12.9	14.4	19.1	25.1	29.3	34.2	31.7	30.9	28.5	25.3	18.4	13.5	6.9285*
	Avg. Relative Humidity (%)	74	76.5	71.5	59.5	63	43.5	72	74	73.5	75	75.5	79.5	21.591*
	Rainfall (mm)	1.3	20.3	126.3	58.5	40.2	41.6	178	189	235.1	25.3	18	0	2.42417
	Presence of infection (%)	14	12	42	52	68	62	56	56	48	26	18	24	

Note: F critical = 4.30095, \* indicates the P value is significant at 0.05.

**Table 4.** Degree of strongylosis infection associated with various risk factors.

Risk factor	No of positives	Degree of infection			X <sup>2</sup> value
		Low	Moderate	Massive	
Sex	Mares	491	147 (29.9%)	166 (33.8%)	59.6778*
	Geldings	482	236 (49.0%)	168 (35.0%)	
Age (years)	1-5	304	72 (23.6%)	98 (32%)	86.3853*
	6-10	387	40 (10.3%)	135 (35.0%)	
	11-15	209	63 (30.1%)	82 (39.3%)	
	16-20	73	36 (49.3%)	22 (30.1%)	
Season	Winter	154	72 (46.8%)	54 (35.1%)	53.7036*
	Spring	159	60 (37.7%)	41 (25.8%)	
	Summer	365	73 (20.0%)	161 (44.1%)	
	Autumn	169	51 (30.2%)	55 (32.5%)	

Note: \* indicate that results are significant at P = 0.05.



In the present study it was noted that animal of either sex was equally affected as 40.91 percent presence was reported in geldings and 40.16 percent in mares. Similar results have also been reported by Saeed et al. (2010). Kvysgaard et al. (2011) conducted a study to determine the presence of strongyle infections in working horses during the dry months in Nicaragua and determined a very high percentage (94%) of strongyle parasites.

Based on the age wise presence analysis, the horses of all age groups showed almost equal presence of disease and there was no significant difference in presence percentage. This advocated that as per the ubiquitous nature of strongylosis, horses of all ages could acquire the infection. However, the younger animals may display aggravated disease condition (Mohammed et al., 2016). Gasser et al. (2004) described that older horses are often observed to have arterial lesions without a history of specific signs, although signs detected in field cases can be correlated with findings at necropsy. The study conducted by Upjohn et al. (2010) in New Zealand also presented no evidence of a significant age effect over the presence of strongylosis among horses. This was also evident in the findings of Saeed et al. (2010), however in contrast we revealed that the intensity of shedding the eggs was not affected by the age of the animals. The current investigation disclosed that age and sex of the animal has no role in the presence percentage of strongylosis at Punjab province.

Overall presence of strongylosis at Punjab province was recorded maximum (67%) at mean temperature 31.2 °C, mean relative humidity 64.62 % and 38.6 mm total rain fall. The minimum presence (12.5%) was recorded at mean temperature 13.6 °C, mean relative humidity 73.37 % and at a total rain fall 19.62 mm. Hutchinson et al. (1989) described that hot dry spring weather (pre-wet season) was the most unfavorable for larval development, migration and survival. Our findings projected that hot / humid environment with moderate rain fall favors presence of the disease as these conditions are best suited for the survival and development of free-living stages of strongyle eggs and larvae outside the host. The low temperature, high percentage of relative humidity and heavy rain fall cause decrease in presence percentage as these conditions do not suite the development / survival of strongyle eggs / larvae. This was also in accordance with Hutchinson et al. (1989) and Sipra et al. (1999).

The degree of infection was categorized into low, moderate and massive forms based upon faecal egg output for gender, age and seasons. A significant variation was observed for the severity of infection with the higher infection noticed in females. The results are in agreement with other reports as well (Maqsood et al., 1996). This may be the case because females are more susceptible to parasitism during pregnancy and per-parturient period due to high stress levels and low immunity. The younger horses (aged 1 to 10 years) were also found out to be suffering from massive infection in this study since they lack the strong immunity developed by adults (aged 11 to 20 years) over time. The adults acquire this solid immunity after the primary and subsequent infections.

The continuous exposure to the acquisition of infective larvae by the adults can stimulate satisfactory immune responses and therefore results in light infections with almost no clinical illness (Lemma and Abera, 2013). High presence with severe infection was recorded during hot rainy seasons which is in line with Nginyi et al. (2001) and Keyyu et al. (2005). These weather conditions play a significant role in larval development and disease transmission (Mohammed et al., 2015).

A control programme can be recommended based on observations made from collected data. Primarily, after completing the somatic migration larvae of large strongyles take another 6 – 8 weeks to reach sexual maturity. Furthermore, the prepatent period of strongyles ranges from 9 to 12 months (Requena-Méndez et al., 2013). The presence is throughout the year, maximum in summer, minimum in winter, not affected by the age and sex of animal and favored by hot/humid environment (Uslu and Guclu, 2007). This would lead to heavy economic losses at national level. The control measures taken to recede the potential adverse effects could be effectual if implemented properly. Horses must be dewormed with broad spectrum dewormer like Piperazine adipate, Ivermectin or moxidectin at least twice a year in May/June and November/December (Boersema et al., 1996). Deworming in early summer would ensure expulsion of worms before reaching sexual maturity and deworming in early winter would ensure the expulsion of remainders of summer (Herd, 1993). In broad terms, it is necessary to avoid treatments of strongylosis during the peak winter months and during peak summer months to retard the development of anthelmintic resistance. Deworming agents should be used alternatively to reduce chances of anthelmintic resistance (Nielsen et al., 2007). For awareness of breeders a campaign on prevention of strongylosis may be launched through electronic and print media by the Livestock department.

### ACKNOWLEDGEMENTS

This research was supported by grants funded by University of the Punjab, Lahore Pakistan.

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