Detection And Distribution Of Gastrointestinal Nematodes In Cows And Buffaloes And Their Fecal Egg Count Per Gram For Parasitic Burden In KPK, Pakistan

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Abstract
The GIT parasites are the main constrain for the decrease productivity of the cattle and buffaloes. The current study was conducted to evaluate the detection and distribution of gastrointestinal nematodes in cows and buffaloes and their fecal egg count per gram for parasitic burden in Khyber Pakhtunkhwa, Pakistan. In the current study, a total of 330 samples were examined. The overall prevalence of gastrointestinal nematodes was 42.12%. The rate of infection in cows was noted to be higher 44.45% than in buffaloes 39.33%. The gastrointestinal nematode infections were lower in female than male animal i.e. 40.76% and 47.14%, respectively. The result regarding gender was noted to be similar for both cows and buffaloes. The young animals were recorded to be more susceptible to gastrointestinal infection with nematode parasites 46.15% as compared to adults 39.50%. Moreover, the prevalence of gastrointestinal Nematodes was higher in months of July-august followed by May-June, September-October and March-April. The prevalence in grazing animals was higher 58.99% than stall feeding 41.01%. Among the nematode species *Trichostrongylus* species were more common followed by *Haemonchus*, *Ostertagia*, *Bunostomum*, *Toxocara*, *Strongyloid*, *Nematodiurus*, *Trichuris* and *Coopeia*, i.e. 14.84%, 10.90%, 9.39%, 8.18%, 7.27%, 6.66%, 4.24%, 3.93% and 3.03%, respectively. The overall egg per gram was 2.01. The analysis of egg per gram showed high value for buffaloes 2.10 than cows 1.89. The young animals were found with more parasitic burden of 2.26 eggs per gram than 1.77 eggs per gram in adults. The grazing animals were also noted to be at great risk of being infected with a high load of parasites as compared to stall fed i.e. 2.30 egg per gram and 1.67 eggs per gram, respectively.

Keywords: Prevalence, Nematodes, Egg per Gram, cattle,

1. INTRODUCTION

Livestock play a crucial role in Pakistan's economy. In the financial year 2013–14 (PES, 2013–14), the value of Pakistan's livestock sector contributed 55.9% of all agricultural goods while making up 11.8% of the country's Gross Domestic Product (GDP) (Jabbar et al., 2015).

Gastrointestinal parasites are the most important parasites which disturb the productivity by reducing milk and meat production of animals by causing many diseases (Artis, 2006) (Ibrahim et al., 2013) (M. K. Khan et al., 2013).These parasites are typically single-host endo-parasites that are dependent on both their host and their environment. The parasitic stages of these nematodes (i.e., the fourth phase larvae and adults) abrade the gastrointestinal tract (GIT) of their host, altering its physiology and negatively affecting the animal's performance and well-being. (Sutherland & Scott, 2010).

In Pakistan gastrointestinal parasites are the main problem which affects the health status and production performance of cattle by causing many parasitic diseases (Shah, Khan, & Rahman, 2017). Parasitism caused by nematodes leads in to the loss of host protein mainly plasma and commonly RBC'S i.e. red blood cells, epithelial cells and mucus and also affect digestion of host, use of nitrogen and energy in the infected animal and also decrease the performance of the host and feed eating (Simcock, Scott, Przemeck, & Simpson, 2006).

2. METHODOLOGY

2.1 Study Area

This study was conducted in different tehsils including Katlang, Takhtbhai, Mardan, Tangi, Shabqadar, Charsadda and Dargai areas. The summer season is extremely hot and high temperature has been observed from June to August. The temperature reaches to its maximum (i.e. above 40°C) in the month of June. The coldest months are December and

January in which the temperature falls to the average 2.09°C. Maximum of the rainfall occurs in July, August, December and January.

2.2 Sample Collection
Fresh fecal samples were collected randomly from rectum of cows and buffaloes from different tehsils of KPK, Pakistan in a separate polythene bags which was tightly packed to avoid air entrance. Total of 330 fresh fecal samples were collected randomly.

2.3 Labeling the sample
After the collection of the fecal samples, they were labeled with animal type, age, sex, date and place of collection with the help of questionnaire which is design for sample record. The fresh fecal samples were carried to the Parasitology lab of Abdul Wali Khan University Mardan (AWKUM).

2.4 Techniques
For further processing fecal samples were passed through different techniques like Zinc sulfate centrifugal floatation technique, Sodium Chloride centrifugal floatation technique, Wisconsin sugar floatation method, Sedimentation techniques and Micrometry to see and identify the parasites present in the fecal samples. Identification of the parasite eggs or cysts made on the basis of morphology and size of eggs. Morphological keys and atlas was used to identify the parasitic eggs (Foreyt, 2013).

2.5 Statistical Software
The data was analyzed using statistical software SPSS (version 16).

3. RESULTS
Out of 330 fecal samples the overall prevalence of gastrointestinal (GIT) nematodes was 42.12% (139/330). In buffaloes (59/150) 39.33%, In cows (80/180) 44.45% (Table 3.1). A slightly higher prevalence was recorded in cows as compared to buffaloes. **T**he prevalence rate in male cattle was 47.14% (33/70) in male and 40.76% (106/260) in female (Table 3.2).

The overall prevalence rate of GIT nematodes was noted to be higher in calves of cows and buffaloes as compared to adults i.e. 46.15% (60/130) in calves and 39.5% (79/200) in adults. The calves of buffaloes showed higher susceptibility to GIT nematodes 45% (27/60) than the cow's calves 35.55%(33/70), (Table 3.3). The higher prevalence in buffalo's calves was not statistically significant ($P<0.05$).

The samples collected during different months of the year showed significant difference ($P<0.05$) in the percent prevalence of GIT nematode parasites. Highest prevalence of GIT parasite was found in the month of July-August 61.25%, followed by May-June 40%, September-October 34.44% and March-April 33.75% (Table 3.4). Higher prevalence 58.99% was recorded in cattle having grazing habit as compared to those reared in farms or in houses 41.01% (Table 3.5). However, the effect of eating habit was statistically significant ($P<0.05$).

Among the nematodes genera the following species like *Haemanchus contortus, Trichostrongylus colubriformis, Ostertagia ostertagi, Toxoxcara vitolurum, Bunostomum phlebotomum, Strongyloid papillosus, Nematodirus spathiger, and cooperia* species were the most common nematodes identified. The nematode parasites are more prevalent in different tehsils of KPK, Pakistan. *Trichostrongylus colubriformis* were the most common with 14.84% and *cooperia* species were the least prevalent with 3.33% in cows and buffaloes of the area. Relative percentage of all the species are in (Table 3.6).

In cows, higher prevalence of *Trichostrongylus colubriformis* 14.45% was recorded, followed by *Haemonchus contortus*, *Bunostomum phlebotomum*, *Ostertagia ostertagi*, *Toxocara vitolurum*, *Stongyloid papillosus*, *Nematodirus spathiger* and *Cooperia*, 12.23%, 9.45%, 8.89%, 8.34%, 7.22%, 5.00% and 3.34% respectively. While in buffaloes a different pattern of specie wise prevalence was recorded i.e. *Trichostrongylus colubriformis*, *Ostertagia ostertagi*, *Haemanchus contortus*, *Toxocara vitolurum*, *Bunostomum phlebotomum*, *Strongyloid pappilosus*, *Nematodirus spathiger* and *Cooperia*, 15.33%, 12.00%, 11.34%, 7.33%, 7.33%, 6.67%, 4.66% and 3.33% respectively (Table 3.7). Moreover, the percent mixed infection in buffaloes was 22.67% and cows 17.22%.

The parasitic burden was noted to be higher in buffaloes (2.10) as compared to cows (1.89), however, the effect of animal type on parasitic burden was not statistically significant ($P>0.05$). While age of the animal was highly significant ($P<0.05$) on parasitic burden and the result showed mean egg per gram (2.26) and (1.77) in young and adult, respectively. While the effect of gender on parasitic burden was more in male (2.09) mean egg per gram then female (1.96). However the effect of living condition on parasitic burden was higher in animals lived in herd and result showed (2.06) mean egg per gram as compared to animals which lived single (1.90) mean egg per gram and the effect of eating

habit was highly significant (P<0.05) on parasitic burden and the result showed more (2.30) mean egg per gram in grazing animals as compared to those animals which were given the forage (1.67) mean egg per gram (Table 3.8).

4. DISCUSSION

The current finding for overall prevalence of GI nematodes was higher than 33.99%, reported by (Ramzan et al., 2017). While (Afridi, Khan, Zaman, Ullah, & Habibullah, 2007) reported nearly same result for overall prevalence i.e. 39.82% and 46.6% respectively. The cattle of Nigeria are also found to be more prone to GI nematodes 71.54% (Adedipe, Uwalaka, Akinseye, Adediran, & Cadmus, 2014). (Kobak & Pilarczyk, 2012) showed 33.1% infection of GI nematodes in Buffaloes. Higher prevalence was recorded in buffaloes 91.44% by (Khalil-ur-Rehman, Tunio, Kuthu, & Sciences, 2009). In contrast to the current study GI infections were higher in buffaloes 39.82 and cows 33.68% (M. N. Khan, Sajid, Khan, Iqbal, & Hussain, 2010). The lower rate of prevalence of GI parasites as compared to the previous studies could be correlated well with the use of anthelminthic drugs and good management condition in the area. While the difference in prevalence of GI parasite between cows and buffaloes in the present and other studies involve management practices, feeding and environmental conditions.

The current study agrees with the findings of (Raza et al., 2013) who reported higher prevalence of GI parasites in male 91% than female 35% (Ameen, Adedokun, Akinola, & Research, 2015) also reported higher prevalence in male than female cattle. In another study the prevalence of GI parasites of cattle was higher in female as compared to male 45.41% and 34.73% respectively (Maharana, Kumar, Sudhakar, Behera, & Patbandha, 2016).

The reason for higher prevalence of GI nematode parasites in male as compared to female is due to the fact that farmer communities pay little attention to the health and nourishment of the male because male has little economic value in the herd.

In the current study the overall prevalence of GIT nematodes were in line with the findings of (Haque et al., 2011), they reported 37.97% in adults and 67.81% in calve in a combine study of cattle and buffaloes. (Raza et al., 2013) also reported higher prevalence in young as compared to adults i.e. 77% in young and 34% in adult cattle. The study of (Bilal, Hameed, & Ahmad, 2009) also reflects a contrasting result of lower prevalence in young cattle as compared to young buffalo's i.e. 56.25% and 75%, respectively. (Amir, 1994) also reported similar results. The current study agrees with the findings of (Raza et al., 2013) and (Marskole, Verma, Dixit, & Swamy, 2016) who reported the higher prevalence of GIT parasites in young cattle as compared to young buffaloes, i.e. buffaloes 74%, cows 77% and buffaloes 62.50%, cows 66.67% , respectively.

The higher rate of infection in young as compared to adult animal correlate well with the fact that young animals have little exposure to the environment and hence lesser adoptability to the environment in terms of acquired immunity, which leads to lack of resistance to the potential pathogen of the environment. The difference in the rate of infection between calves of cows and buffaloes in the present and previous studies is negligible. The higher or lower prevalence in an animal type may be attributed to the management practices under taken in that area.

The current study was in line with the results of (Nath et al., 2016) they reported higher prevalence in the month august followed by April. The prevalence of GIT parasites was higher in summer season as compared to winter season (Saha, Bhowmik, & Chowdhury, 2013). Another study recorded showed higher prevalence in the month of June followed by March and September-October i.e. 58.79%, 53.77% and 39.65%, respectively (Nnabuife et al., 2013). (Mamun, Begum, & Mondal, 2011) also reported higher prevalence in rainy season 71.17% than the winter season 57.27%. Contrary to the current results, (Fawzi & Elsohaby, 2017) reported higher prevalence in winter 25.6% than in summer 10.3%.

The current study and most of the previous studies revealed higher prevalence of GIT parasites in summer season, especially in the months with high temperature and humidity level. The hot and humid condition during the month of May-June and July-August provides suitable environment for the survival and development of parasitic eggs, larvae and cysts.

In the present study the significant effect of eating habit agrees with the findings of (Rajakaruna & Warnakulasooriya, 2011) the prevalence of parasites were double in grazing cattle as that of non-grazing or stall feeding. (Bilal et al., 2009) also reported significant correlation between eating habit and prevalence of GIT parasites in both cattle and buffaloes. The higher prevalence was found in the animal with grazing habit as compared to those having stall feeding eating habit. The infected cattle release the parasite's eggs in the environment when they defecate. The presence of eggs and other infective forms of these parasites in the pasture increase the potential risk for a grazing animal to get infected.

The current findings were in line with the study of (Afridi et al., 2007) in which high prevalence of *Trichostrongylus* followed *Haemonchus* 21.08%, 16.30%, respectively and lower prevalence of *Trichiuris* 3.86% was reported. (Rafiullah, Sajid, Shah, Ahmad, & Shahid, 2011) also reported high prevalence of *Trichostrongylus* 13.83% followed by *Nematodirus* 6.58% and a low prevalence of *Haemonchus contortus* 2.76%. In another study in Pakistan, *Haemonchus* was reported highly distributed specie of nematode by *Ostertagia* species, while cooperia was noted rare. The current

results also agrees with the findings of (Adem, Anteneh, & Health, 2011) in Ethiopia, they reported *Trichostrongylus* to be the more prevalent genera among Nematodes, followed by *Haemonchus*, *Oesophagostomum*, *Cooperia* and *Trichuris*, 37%, 11.6%, 11%, 2.3% and 1.2%, respectively. The *Ostertagia* was shown to be highly prevalent followed by *Haemonchus* in Balochistan (Ramzan et al., 2017). The *Strongyloids* and *Trichuris* were more common as compared to *Haemonchus* and *Ostertagia* in Ethiopia (Muktar, Belina, Alemu, Shiferaw, & Belay, 2015).

The high prevalence of *Trichostrongylus* and *Haemonchus* in the current and previous studies in the tropical and subtropical areas could be attributed to the hot and humid condition which is favorable for the free living stages of above mentioned species.

Higher mean eggs per gram 3.21, as compared to the current finding was reported by (Nath et al., 2016) in cattle. (Fawzi & Elsohaby, 2017) reported higher mean value for eggs per gram 2, than our results. The parasitic burden in young was noted to be higher in young as compared to adults in the current as well as previous studies (Ramzan et al., 2017) reported higher average eggs per gram in young 2.08 than adults 0.9 in cattle and buffaloes. (Bachal, Sharif, Rahamatullah, & Aijaz, 2002) also reported high parasitic burden in young. The gender wise analysis of parasitic burden in the current study was inn contrast with the findings of (Ramzan et al., 2017) they reported lower eggs per gram in male 2.17 than 2.35 in female buffaloes. The parasitic burden was lower in male as compared to female 1 epg and 1.13 epg, respectively. Comparison of eggs per gram showed high value in young than in adults (Karim, Farajallah, & Suryobroto, 2016).

The association between animal type and parasitic burden could be attributed to the difference in the management practices and feeding habits of these two groups. More over the higher prevalence in young than in adults correlates well with fact that young are given little attention as compared to adult and their lack of resistance to the potential pathogens. The higher egg per gram is due to the repeated exposure of grazing animals to the contaminated environment at the pasture. The cattle in herd are at more risk for GIT infections as compared to the cattle reared singly, because single infected cattle in the herd could cause infection to many of others by releasing hundreds and thousands of eggs in their fecal material to the environment.

Tables:

Variable	Cows	Buffaloes
Frequency	180	150
No. of infected	80	59
Percent infection	44.45%	39.33%

Table: 3.1 Prevalence of Gastrointestinal nematodes in Cows and Buffaloes

Variable	Male	Female	Buffalo Male	Buffalo Female	Cow Male	Cow Female
Frequency	70	260	30	120	40	140
No. of infected	33	106	14	45	19	61
Percent infection	47.14%	40.76%	46.66%	37.50%	47.50%	43.57%

Table: 3.2 Gender wise prevalence in both Cows and Buffaloes

Variable	Young	Adult	Buffalo calves	Buffalo adults	Cow calves	Cow adults
Frequency	130	200	60	90	70	110
No. of infected	60	79	27	32	33	47
Percent infection	46.15%	39.5%	45.0%	35.55%	47.14%	42.72%

Table: 3.3 Age wise prevalence in both Cows and Buffaloes

MONTH	No. of fecal samples examined	No. of positive samples	Prevalence rate %
March-April	80	27	33.75%
May-June	80	32	40.00%
July-August	80	49	61.25%
September-October	90	31	34.44%

Table: 3.4 Month wise prevalence of GI nematodes

Eating Habit	Infection		Total	Prevalence Rate %
	Yes	No		
Grazing	82	70	152	58.99%
Forage	57	121	178	41.01%
Total	139	191	330	100.00%

Table: 3.5 Prevalence of Gastrointestinal nematodes based upon Eating Habit

Species of Nematodes	Number of Fecal Samples Examined	Number of Fecal Samples Positive	Percent Infected
Trichostrongylus colubriformis	330	49	14.84%
Haemanchus contortus	330	39	11.81%
Ostertagia ostertagi	330	34	10.30%
Bunostomum phlebotomum	330	28	8.48%
Toxoxcara vitolurum	330	26	7.87%
Strongyloid papillosus	330	23	6.96%
Nematodirus spathiger	330	16	4.84%
Cooperia	330	11	3.33%

Table: 3.6 Distributions of Various Gastrointestinal Nematodes

Species of Nematodes	Cows	Buffaloes
Trichostrongylus colubriformis	26/180; 14.45%	23/150; 15.33%
Haemanchus contortus	22/180; 12.23%	17/150; 11.34%
Ostertagia ostertagi	16/180; 8.89%	18/150; 12.00%
Bunostomum phlebotomum	17/180; 9.45%	11/150; 7.33%
Toxoxcara vitolurum	15/180; 8.34%	11/150; 7.33%
Strongyloid papillosus	13/180; 7.23%	10/150; 6.67%
Nematodirus spathiger	09/180; 5.00%	07/150; 4.66%
Cooperia	06/180; 3.34%	05/150; 3.33%
Mixed infection	31/180; 17.22%	34/150; 22.67%

Table: 3.7 Specie wise prevalence of Gastrointestinal nematodes in Cows and Buffaloes

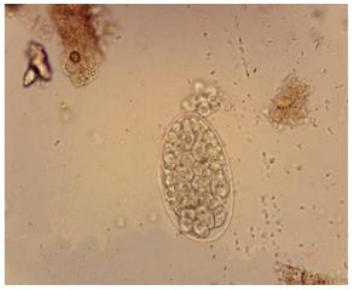
Parameters	Prevalence Rate %	Mean EpG
Cows	44.45	1.89
Buffaloes	39.33	2.10
Young	46.15	2.26
Adult	39.50	1.77
Male	47.14	2.09
Female	40.76	1.96
Single	39.72	1.90
Herd	44.02	2.06
Grazing	53.94	2.30
Forage	32.02	1.67
Overall	42.12	2.01

Table: 3.8 Fecal Egg Count/ Egg per Gram

Micrographs:

Micrograph of *Toxocara vitulorum*

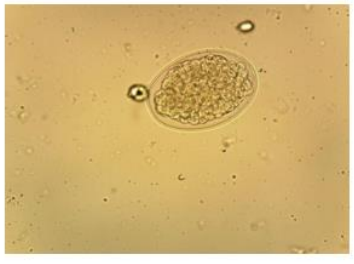
Micrograph of *Ostertagia ostertagi*



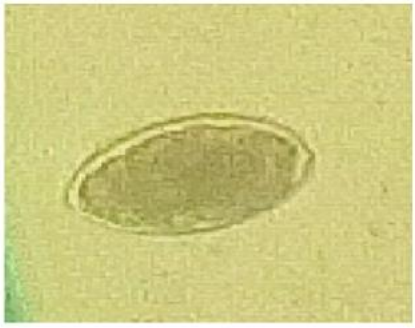
Micrograph of *Trichostrongylus colubriformis*

Micrograph of *Nematodirus spathiger*

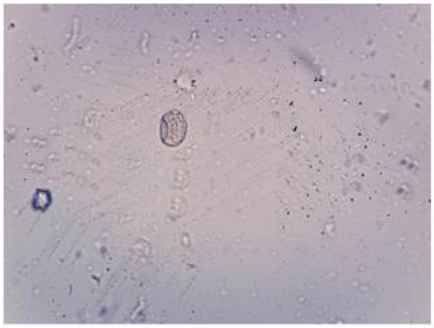
Micrograph of *cooperia* spp.



Micrograph of *Bunostomum phlebotomum*



Micrograph of *Haemonchus contortus*



Micrograph of *Strongyloid pappilosus*

5. CONCLUSION

The current study shows that the prevalence of parasite and their subsequent parasitic burden was high in the grazing cows and buffaloes which reflect lack of management practices in the area. The higher prevalence of GI nematodes in male as compared to female indicates that the males are normally ignored by farmer due to their little profitability as compared to female in the herd. The high prevalence of *Trichostrongylus* and *Haemonchus* species shows that the climatic and environmental condition of the area is suitable for the survival of their free living forms of life cycle. The higher prevalence in the months of July-August reveals that the hot humid conditions are suitable for the life cycle of GI nematodes

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