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## Epidemiology of different gastrointestinal helminths in buffaloes in relation to age, sex and body condition of the host

**Farrah Deeba, Anas Sarwar Qureshi, Abdul Rehman Kashif and Ijaz Saleem**

**Abstract**

Buffaloes (*Bubalus bubalis*) are a major source of cash for many rural populations, especially in semi-arid and arid regions of developing countries. This is considered as “black gold” of Pakistan. Even well managed animals harbor gastrointestinal parasites. Parasitic diseases are important because they cause high economic losses. The present study was conducted from October 2014 to January 2015 in different tehsils of district Khushab, Pakistan to determine the epidemiology of different gastrointestinal helminths in buffaloes in relation to age, sex and body condition of the host. Fecal samples of 369 buffaloes were examined. Data was subjected to statistical analysis through using chi square test and analysis of variance using SAS software. Statistical analysis revealed that 201 samples were found positive and prevalence of different parasites were recorded as 40.92%. The parasites were *Toxocara vitulorum*, (18.54%) *Fasciola*, (17.21%) *Paramphistomum cervi*, (6.62%), *Oesophagostomum radiatum*, (12.44%) *Haemonchus contortus*, (15.26%) *Trichuris*, (1.98%) *Bunostomum* (5.96%) and *Ostertagia* (17.88%). Age wise prevalence was 32.03% and 46.34% in different age groups (>2 years and < 1 year) of buffaloes calf and adult buffaloes, respectively while sex wise prevalence was 42.5% and 39.71% in male and female buffaloes, respectively. Body condition wise prevalence was 23.63%, 36.34% and 58.99% respectively, in different groups (good, normal and poor). The study concluded that the prevalence of gastrointestinal parasites in buffaloes is very common in the study area irrespective of age, sex and body condition of the host. Usage of proper anthelmintics is strongly recommended to control the infections and to enhance buffalo productivity.

**Keywords:** Buffalo, gastrointestinal helminths, age, sex, body condition

**Introduction**

In Pakistan buffalo (*Bubalus bubalis*) is one of the most important species of domestic livestock and plays important role in the rural economy of the country. In contrast to the statement ‘an asset undervalued’ (FAO, 2004) [1] the majority of small and marginal farmers depend on buffaloes for their livelihood as they also serve as an insurance against the risk of crop failure due to natural calamities (Sreedevi and Hafeez, 2014) [2]. Epidemiological survey of parasitic infections is an important aid to combat infections more effectively and in controlling economic losses by adopting effective control measures. Helminth infections of water buffaloes cause huge economic losses in a variety of ways i.e. low fertility, reduced work capacity, involuntary culling, reduced feed intake, low milk production, treatment costs and mortality (Raza *et al.*, 2012) [5]. Prevalence of gastrointestinal helminths in ruminants has been reported from 25 to 92% in different areas of Pakistan at different times (Raza *et al.*, 2007; Kakar *et al.*, 2008; Raza *et al.*, 2012) [7, 8, 5]. Various risk factors which include age, sex, season, influences the prevalence of gastrointestinal nematodes in food animals as stated by Ramzan *et al.* (2017) [4]. A better understanding of the epidemiology of helminths is essential for a successful parasite control program. So the present study was undertaken to determine the prevalence of gastro-intestinal tract helminths in relation to age sex and body condition in buffaloes (*Bubalus bubalis*) of district Khushab (Punjab), Pakistan.

**Materials and Methods****Study area**

District Khushab has extreme hot and cold climate. Its land comprises mainly hills, plateaus, plains and deserts with river Jhelum flowing on its East. Some of the areas of tehsil Khushab

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are low lying and get flooded in rainy season. Selection of the areas was made on the basis of reported district buffaloes population in Livestock Census (Anonymous, 2013) [9]. For making the study more meaningful and demographically diverse, Tehsils of district Punjab were randomly selected (Khushab, Noorpur Thal, Quaidabad, Sub-Tehsil Naushehra). This study period spanned over 4 months.

### Study populations

A total of 369 buffaloes (*Bubalus bubalis*) of either sex and all ages were selected randomly without any distinction of diseased or non-diseased animals. Sample size was calculated by keeping the 59.38% (Zaman *et al.*, 2014) [10] expected prevalence with 95% confidence limits and 5% desired absolute precision. Sample size was calculated as described by (Thrusfield, 2007) [11]. Animals were divided in to three groups adult (>2yrs.), heifers (1-2yrs.) and calves (<1 yr.)

### Sample collection and preservation

Faecal sample (3-5g) were directly collected in clean plastic containers from the rectum or the top of freshly passed deposit from the soil. Samples were stored at 4 °C until they were being processed (Soulsby, 1982) [3]. Formalin was used as preservative in ratio of 3:1(3 parts formalin and 1 part faecal sample).

### Sample analysis

Fecal samples were examined for helminthes by using direct and indirect techniques. Both Soulsby (1982) [3].

#### Direct Method

One gram of fecal sample was mixed well in a drop of water and a relatively homogenous and transparent preparation was obtained and examined under microscope by placing a drop of suspension on slide with cover slip. At least three direct smears were examined from each sample.

In order to cope with the false negative results we also applied the indirect method or concentration techniques, i.e. floatation and sedimentation.

#### Indirect Methods

##### Floatation method

**1. Simple floatation:** One gram of sample was taken in a beaker and mixed with distilled water. Then this mixture was sieved through a fine muslin cloth or sieve. The filtrate was then mixed with 4-5 ml of saturated salt solution. The test tube was filled up to its brim and a cover slip was placed. Coverslip was placed on slide and after 30 minutes examined under microscope at 10X for presence of helminthes eggs.

**2. Centrifugal Floatation:** One gram of sample was mixed in distilled water and then filtered through a fine sieve. The filtered material was then mixed with saturated salt solution in a ratio of 1:3 and centrifuged at 1500 rpm for 5 min. Then the superficial contents were transferred on a clean and dry glass slide and examined under the microscope.

##### Sedimentation method

**1. Simple Sedimentation:** Five gram of sample was mixed in tape water. The suspension was strained through the cheese cloth into a conical flask. It was left to be settled for 20-30 min. Supernatant was decanted and washing process was continued until the supernatant was clear. Then a drop was taken from the top layer of sediment with Pasteur's pipette on

microscope slide and examined under the microscope. Same procedure was repeated by taking a drop from the bottom layer of the sediment.

**2. Centrifugal sedimentation:** The procedure was similar to that of simple sedimentation with the addition of centrifugation of sample at 1500-2000 rpm for 5 min.

### Data Analysis

The mean prevalence of each parasite and its percentage was calculated by using appropriate formula (Steel *et al.*, 1997) [12] and data were analyzed by analysis of variance using SAS software.

### Results

According to the livestock census 2009, the total reported population of buffaloes was 168 million heads. The buffaloes were selected randomly irrespective of condition/nutritional status from the buffaloes reared areas of district Khushab. Most of the buffaloes population reared in the rural areas in free range grazing system. The ages of the buffaloes were determined interrogating the farmers or by examining the teeth. The nutritional status of the animals was determined by visual observation. A total number of 369 samples were collected from the four tehsils of selected district and 201 samples were found to be positive for helminths.

Age of the animal had an effect on the prevalence of gastrointestinal parasitic infections. In this study, it was detected that prevalence of gastrointestinal parasitic infections was reasonably higher in adult buffaloes >2years (85.38%,  $P>0.70$ ) and lower in calves. In yearling (>1 year to  $\leq 2$  years) recorded (79.17%,  $P>0.31$ ) and in calves recorded ( $\leq 1$  year) (54.29%,  $P>0.08$ ). (Tab.1; Fig.1) In this study, it was noted that prevalence of gastrointestinal parasitic infections in buffaloes population was pointedly higher in females (78.05%,  $P>0.135$ ) than the male (72.64%,  $P>0.134$ ) host. (Tab.2; Fig.2) In the present study, it was detected that prevalence of gastrointestinal parasitic infections was significantly higher in poor body conditioned host buffaloes (93.75%) than normal body condition buffaloes (55.56%). Poor body conditioned cattle were 3.35 and 12 times more susceptible than medium and normal body conditioned buffaloes, respectively. (Tab.3; Fig.3)

During the study period, 369 samples of buffaloes population were examined through faecal sample examination, of which 201 were found infected with one or more species of gastrointestinal parasites indicating an overall prevalence 54.47%. (Tab.4; Fig.4). In the present study 369 buffaloes were examined through faecal sample examination, of which 201 were found infected with one or more species of gastrointestinal parasites indicating an overall prevalence 54.47%. The helminths identified were the, *Toxocara vitulorum*, (13.93%) *Fasciola hepatica*, (12.94%) *Paramphistomum cervi*, (4.98%) *Oesophagostomum radiatum*, (12.44%) *Haemonchus contortus*, (11.44%) *Trichuris*, (1.49%) *Balantidium coli* (24.88%) *Bunostomum* (4.48%) and *Ostertagia* (13.43%). From this study, it was observed that the prevalence of *Balantidium coli*, (24.88%) whereas *Trichuris*, (1.49%) infections were the lowest. (Tab.5; Fig.5) Age of the host had an effect on the prevalence of gastrointestinal parasitic infections in buffaloes. In this research, it was observed that prevalence of *Fasciola hepatica*, *Haemonchus contortus*, *Trichuris*, *Balantidium coli* and *Bunostomum* was higher in animals of below age 1 year.

*Toxocara vitulorum*, and *Paramphistomum cervi*, infection higher in animals of age higher than 2 years whereas *Oesophagostomum radiatum*, *Balantidium coli* and *Ostertagia* infection higher in animals of age between 1-2 years. (Tab.6; Fig.6). In this study, it was recorded that prevalence of gastrointestinal parasites (*Toxocara vitulorum*, *Fasciola hepatica*, *Oesophagostomum radiatum*, *Haemonchus contortus*, *Trichuris* *Balantidium coli* *Bunostomum* and *Ostertagia* infection was in buffaloes was significantly higher in females than the male buffaloes except *Paramphistomum cervi*. (Tab.7; Fig.7). It was observed that prevalence of gastrointestinal parasitic infections of *Toxocara vitulorum*, *Fasciola hepatica*, *Paramphistomum cervi* *Oesophagostomum radiatum*, *Haemonchus contortus*, *Trichuris* *Balantidium coli* *Bunostomum* and *Ostertagia* were significantly higher in poor body conditioned buffaloes than normal/medium and good body condition animals. (Tab.8; Fig.8).

### Discussion

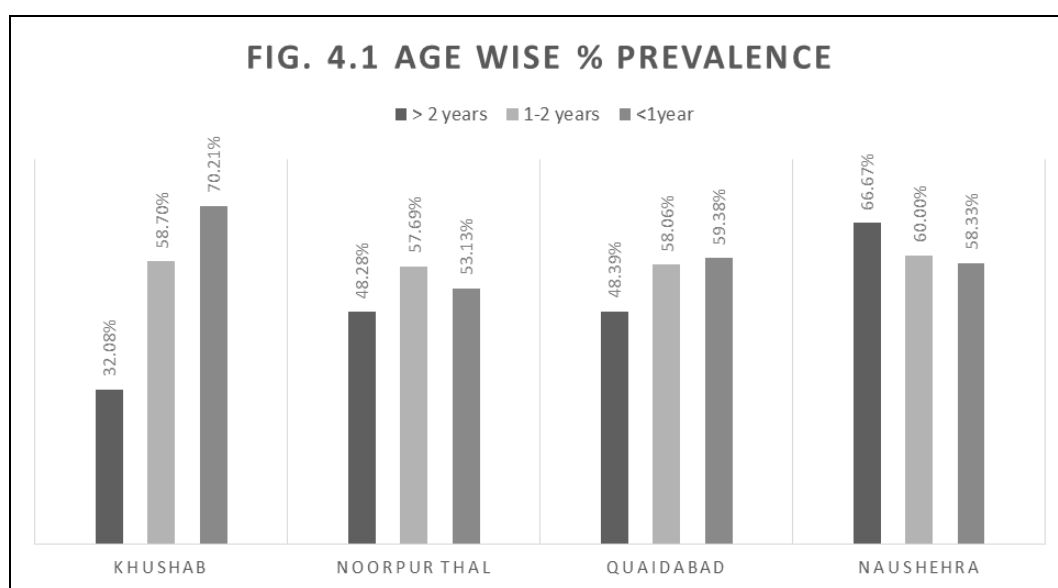
In this study, overall prevalence of gastrointestinal parasitic infections in buffaloes was recorded 54.47% in different areas of the district Khushab, Punjab, Pakistan. This result is related to the results of the Mir *et al.* (2013) [14] who recorded 51.29 % in dairy animals infected with mixed helminths. In the present study the most prevalent parasite was *Toxocara vitulorum*, it was also reported to be the most frequently occurring parasite in buffaloes as stated by Akhter *et al.* (2001). The results are in contrast to the findings made by Zaman *et al.* (2014) [10] (17.75%), and Bhutto *et al.* (2002) [22] (33%) in ruminants. In the present study prevalence of *Fasciola hepatica*, was lower (12.94%) as compared to the prevalence 31.14% reported by Gupta (2002) [16] in animals. Prevalance of *Paramphistomum* was lower (4.98%) as compared to the findings of Mamun *et al.* (2011) [18]; who recorded 29.24%, prevalence. In case of *Oesophagostomum radiatum*, prevalence is much lower than the findings of Guzel and Kozan (2013) [19] who recorded 41.6% prevalence in livestock. Prevalance of *Haemonchus contortus*, (11.44%) was found to be similar to the findings of the Choubisa and Jaroli (2013) [20] who recorded the 11.32% but much higher than the finding of the Lashari and Tasawar (2011) who

recorded 6.5% prevalence in livestock. In case of *Trichuris*, its occurrence found to be lower than the findings of the Bhutto *et al.* (2002) [22] who reported 2 % prevalence in study animals. In the present study the prevalence of *Balantidium coli* (24.88%) is almost close to the findings reported by Bilal *et al.* (2009) [23] who recorded 25% but much lower than the prevalence reported by Roy *et al.* (2011) [24] and Mamun *et al.* (2011) [18] 45.03% and 37.2% respectively in animals. Prevalance of *Bunostomum* was higher as compared to the study conducted by Raza *et al.* (2012) [5] who recorded 1.6% prevalence. In the present study prevalence of *Ostertagia* prevalence (13.43%) was higher in contrast to the study conducted by Afridi *et al.*, (2007) [25] who recorded 9.44% parasite infection in animals.

The prevalence of infection was observed higher in the young calves (61.78%) as compared to the percentage of parasitic infestation in adult animals. The higher infection in young animals than that in older ones may be attributed to lesser resistance because of lesser exposure to different species of helminthes compared with the older animals (Bilal *et al.*, 2009; Raza *et al.* 2012; Zaman *et al.* 2014) [23, 5, 10].

The infection is also related to the body health condition and finding indicates the highest prevalence (93.75%) was recorded in the animals with poor health condition. The findings are in line with Tigist *et al.* (2012) [26] where high helminth load (57.7%) was recorded in animals with poor body condition and in contrast with Regassa *et al.* (2006) [27] who reported that body condition of the animal did not show significant association with prevalence of the parasite. Loss of body condition in the study animals could be due to change of fodder and the presence of other health disorders.

In the present study, it was noted that prevalence of gastrointestinal parasitic infections in buffalo population was higher in females (78.05%), similar findings were reported by Bhutto *et al.* (2002) [22] where prevalence of helminthes was higher in female calves (48.30%) than in male calves 45.12%. It might be assumed that higher prevalence of the parasites in females as compared with males is due to lowered resistance of female animals aggravated by their reproductive events and insufficient/unbalanced diet against higher needs as stated by Raza *et al.*, (2012) [5].



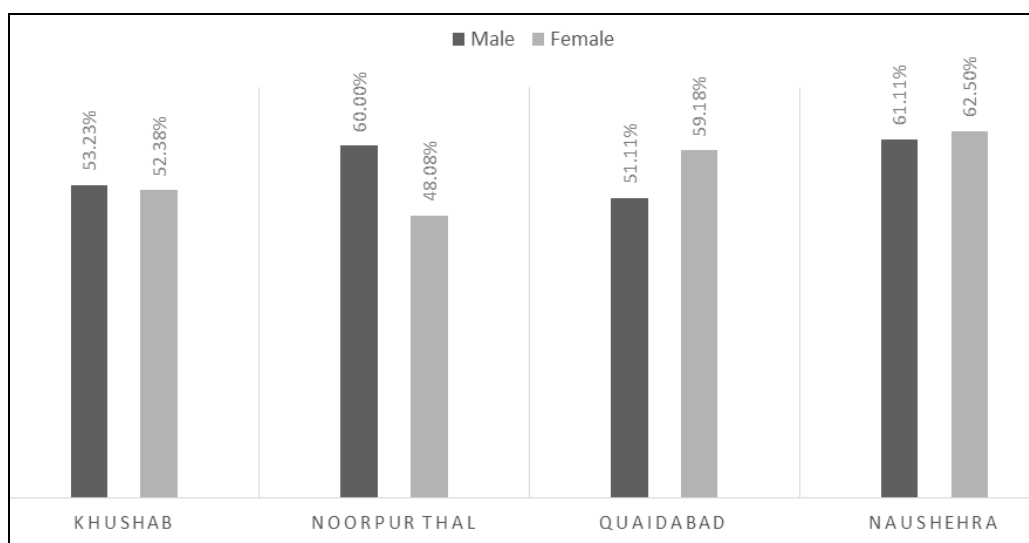
**Fig 1:** Age wise prevalence of helminths

**Table 1:** Age related prevalence in buffalo’s population of district Khushab (Chi-Square P-Value for >2 years is 0.70, for 1-2 years is 0.31 and for <1 year is 0.08).

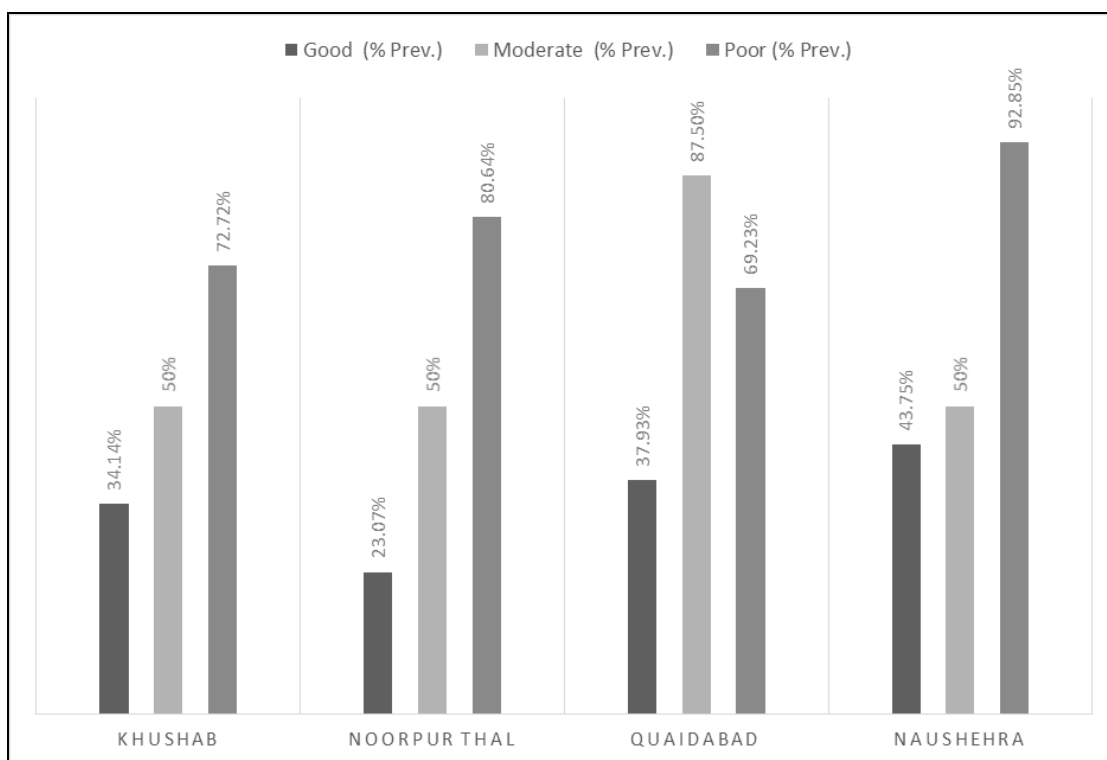
Tehsils	Age wise % Prevalence					
	No of infected animals	> 2 years	No of infected animals	1-2 years	No of infected animals	<1year
Khushab	17/53	32.08%	27/46	58.70%	33/47	70.21%
Noorpur Thal	14/29	48.28%	15/26	57.69%	17/32	53.13%
Quaidabad	15/31	48.39%	18/31	58.06%	19/32	59.38%
Naushehra	10/15	66.67%	9/15	60.00%	7/12	58.33%
Total	56/128	43.75%	69/118	58.47%	76/123	61.78%

**Table 2:** Sex wise prevalence of helminths in buffaloes district Khushab (Chi-Square P-Value for male is 0.134 and for female is 0.135).

Tehsils	No of infected animals	Male	No of infected animals	Female
Khushab	33/62	53.23%	44/84	52.38%
Noorpur Thal	21/35	60.00%	25/52	48.08%
Quaidabad	23/45	51.11%	2/49	59.18%
Naushehra	11/18	61.11%	15/24	62.50%



**Fig 2:** Sex wise prevalence of helminthes in buffaloes in different tehsils of Khushab



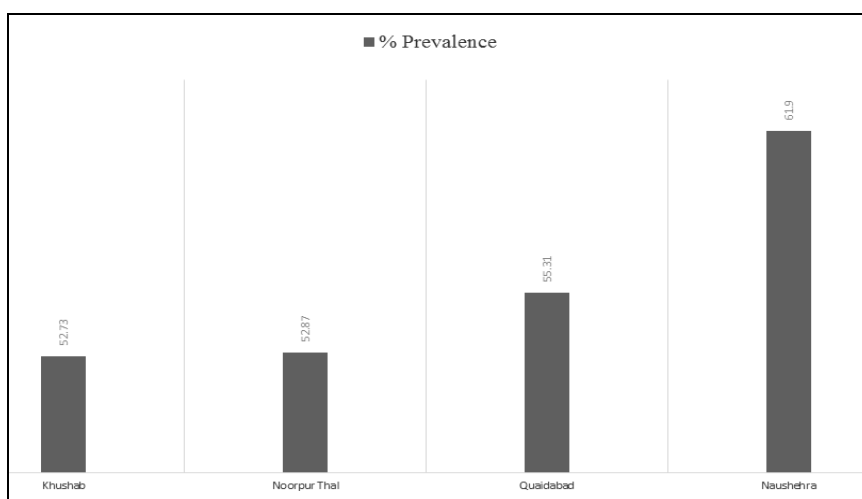
**Fig 3:** Body condition wise prevalence of helminths in buffaloes in different tehsils of Khushab

**Table 3** Body condition related prevalence of helminths in buffaloes in district Khushab.

Tehsils	No of infected animals	Good (% Prev.)	No of infected animals	Moderate (% Prev.)	No of infected animals	Poor (% Prev.)
Khushab	14/41	34.14%	25/50	50%	40/55	72.72%
Noorpur Thal	6/26	23.07%	15/30	50%	25/31	80.64%
Quaidabad	11/29	37.93%	14/26	87.5%	27/39	69.23%
Naushehra	7/16	43.75%	6/12	50%	13/14	92.85%

**Table 4:** Overall prevalence of helminthes in different tehsils in district Punjab %

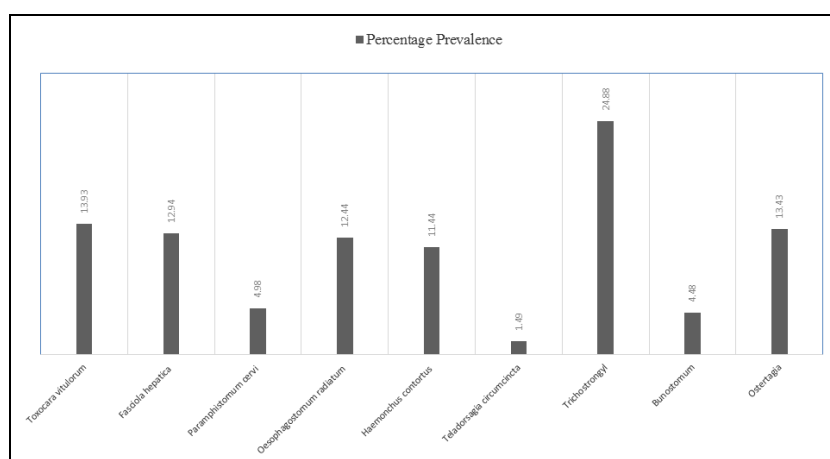
Tehsils	No of infected animals	% Prevalence
Khushab	77/146	52.73
Noorpur Thal	46/87	52.87
Quaidabad	52/94	55.31
Naushehra	26/42	61.90
Overall	201/369	54.47



**Fig 4:** Overall prevalence of helminths in different tehsils of district khushab

**Table 5:** Percentage prevalence of different types of helminths

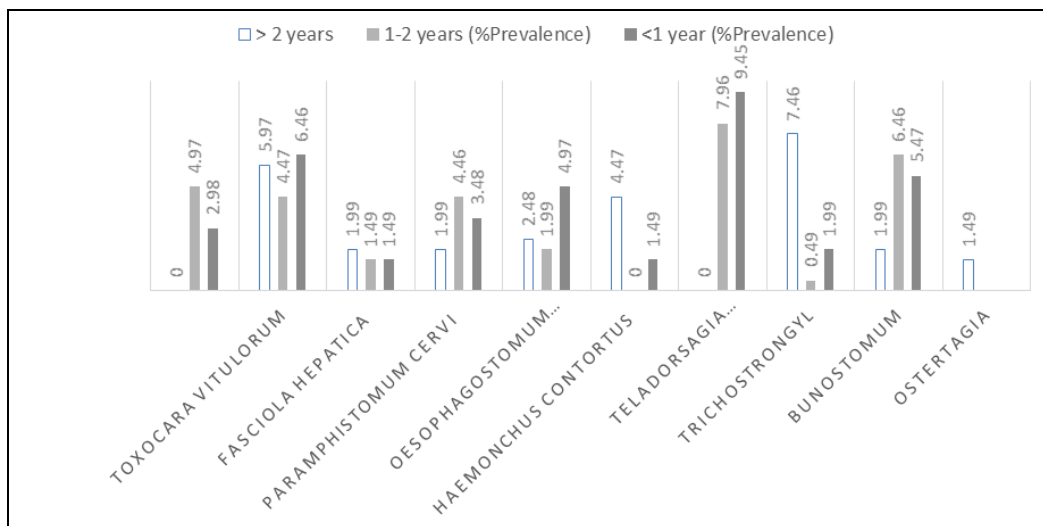
Type of Helminths	Percentage Prevalence
<i>Toxocara vitulorum</i>	13.93
<i>Fasciola hepatica</i>	12.94
<i>Paramphistomum cervi</i>	4.98
<i>Oesophagostomum radiatum</i>	12.44
<i>Haemonchus contortus</i>	11.44
<i>Trichuris</i>	1.49
<i>Balantidium coli</i>	24.88
<i>Bunostomum</i>	4.48
<i>Ostertagia</i>	13.43
Total	100



**Fig 5:** Percentage prevalence of different types of helminths

**Table 6:** Age related prevalence of gastrointestinal parasitic infections in buffaloes.

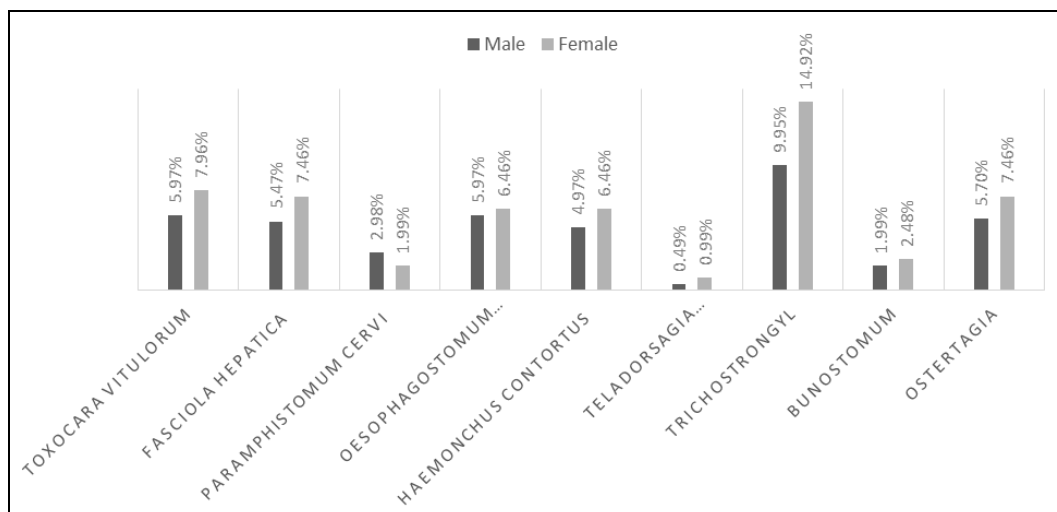
Type of Helminths	% Prevalence		
	> 2 years (%Prevalence)	1-2 years (%Prevalence)	<1 year (%Prevalence)
<i>Toxocara vitulorum</i>	5.97	4.97	2.98
<i>Fasciola hepatica</i>	1.99	4.47	6.46
<i>Paramphistomum cervi</i>	1.99	1.49	1.49
<i>Oesophagostomum radiatum</i>	2.48	4.46	3.48
<i>Haemonchus contortus</i>	4.47	1.99	4.97
<i>Trichuris</i>	0	0	1.49
<i>Balantidium coli</i>	7.46	7.96	9.45
<i>Bunostomum</i>	1.99	0.49	1.99
<i>Ostertagia</i>	1.49	6.46	5.47



**Fig 6:** Age wise prevalence

**Table 7:** Sex related prevalence of gastrointestinal parasitic infections in buffaloes

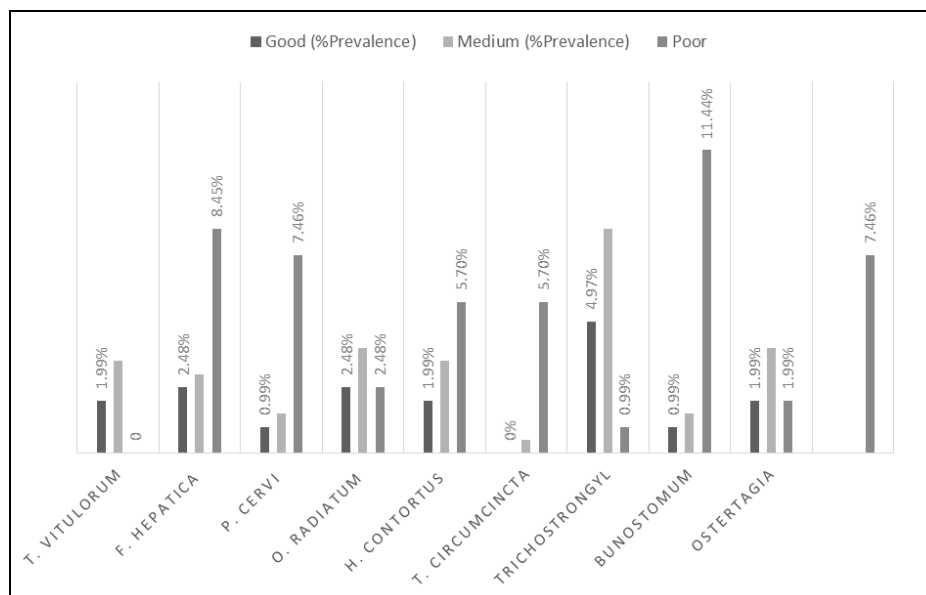
Type of Helminths	Male	%Prevalence	Female	%Prevalence
<i>Toxocara vitulorum</i>	12	5.97	16	7.96
<i>Fasciola hepatica</i>	11	5.47	15	7.46
<i>Paramphistomum cervi</i>	6	2.98	4	1.99
<i>Oesophagostomum radiatum</i>	12	5.97	13	6.46
<i>Haemonchus contortus</i>	10	4.97	13	6.46
<i>Trichuris</i>	1	0.49	2	0.99
<i>Balantidium coli</i>	20	9.95	30	14.92
<i>Bunostomum</i>	4	1.99	5	2.48
<i>Ostertagia</i>	12	5.7	15	7.46



**Fig 7:** Sex Wise Prevalence

**Table 8:** Body condition related prevalence of gastrointestinal parasitic infections in buffaloes.

Type of Helminths	Good (%Prevalence)	Medium (%Prevalence)	Poor (%Prevalence)
<i>T. vitulorum</i>	1.99	3.48	8.45
<i>F. hepatica</i>	2.48	2.98	7.46
<i>P. cervi</i>	0.99	1.49	2.48
<i>O. radiatum</i>	2.48	3.98	5.7
<i>H. contortus</i>	1.99	3.48	5.7
<i>T. circumcincta</i>	0	0.49	0.99
<i>Balantidium coli</i>	4.97	8.45	11.44
<i>Bunostomum</i>	0.99	1.49	1.99
<i>Ostertagia</i>	1.99	3.98	7.46

**Fig 8:** Body condition wise prevalence

## Conclusion

In the present study, overall prevalence of gastrointestinal helminth parasites indicates that gastrointestinal helminthiasis is an important health problem in dairy animals. The gastrointestinal helminths are prevalent in all age groups and either sex (Male and Female) of buffaloes in the study area. The phenomenon of mixed helminth infections has been suggested to be an important cause of reduced production and morbidity in livestock. Keeping in view the above factors, strategic treatment and control programmes may be formulated to control gastrointestinal parasitic infections in buffaloes in and around district Khushab.

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