BIOSCIENCE JOURNAL

EPIDEMIOLOGICAL ANALYSIS OF *Paramphistomum* SPECIES IN RUMINANTS IN SELECTED DISTRICTS OF KHYBER PAKHTUNKHWA (KPK), PAKISTAN

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How to cite: RAFIQ, N., et al. Epidemiological analysis of *Paramphistomum* species in Ruminants in selected Districts of Khyber Pakhtunkhwa (KPK), Pakistan. *Bioscience Journal*. 2023, **39**, e39066. https://doi.org/10.14393/BJ-v39n0a2023-66497

Abstract

This epidemiological study was conducted to compare the climatic conditions of four different districts of Khyber Pakhtunkhwa, Pakistan, in terms of the prevalence of parasitic infection *Paramphistomum* in cows and buffaloes. A total of 2400/cows and buffaloes (200/per month and 50/per district) fecal samples were collected. The samples were examined for *Paramphistomum* egg contamination by direct microscopic examination and sedimentation methods. The infectivity ratio was 17.5% in cows and 17% in buffaloes; the higher rate of infection was recorded in autumn with 41% in cows and 36% in buffaloes. Whereas the lowest ratio was 6.5% in cows and 7% in buffaloes recorded during spring. It was also noted that a high rate of prevalence was recorded in older animals (higher than 2 years of age) than younger (less than 2 years of age), while gender-wise, it was observed that male animals were more affected than females. Prevalence was higher in cows in Mardan district (19.30%) followed by Nowshera (19.10%) and Swabi (17.80%) districts while lowest in Charsadda district (13.80%) where in buffaloes the higher prevalence was recorded in Swabi (18.80%) followed by Mardan (18.60%) then Charsadda (16.80%) while lowest in Nowshera (13.80%).

Keywords: Epidemiology. *Paramphistomum*. Parasitic. Prevalence. Sedimentation.

1. Introduction

Paramphistomosis is caused by a Paramphistomum species (Rafiq et al. 2020; Qureshi et al. 2021) having a worldwide distribution, however widespread in tropical and subtropical regions, predominantly in Australia, Africa, Russia, Asia and Eastern Europe (Rolfe et al. 1991; Ayaz et al. 2013). The gastrointestinal parasites incidence in cattle in different areas of Pakistan has been stated from 25.1 to 92% at different periods (Iqbal et al. 1993, Ali et al. 2000; Raza et al. 2007; Ijaz et al. 2008; Al-Shaibani et al. 2008; Kakar et al. 2008).

In the world, some countries measured this parasite as a key restriction on production. In the same way, *Paramphistomum* infections *(e.g., P. cervi, P. microbothrium, and P. ichikawai*) have the same allocation, mostly in Asia. The rates of prevalence in Asia (30-60%) are still noted in some areas (Raza et al. 2009).

Paramphistomosis is a significant endoparasitic disease of ruminants with great economic loss to livestock (Sivajothi et al. 2014; Rafiq et al. 2020; Qureshi et al. 2021). The main source of severe injury to the lining of the intestine (enteritis) is immature flukes, and especially in young animals, which effect

dehydration, maldigestion, and death. *Paramphistomum* causes low diet exchange, which consequences in weight loss reduce fertility, decrease milk production, necrosis, polydipsia, anorexia, which ultimately results in great finical loss (Horak et al. 1971; Rolfe et al. 1991; Rangel et al. 2003).

The present study was designed to record the epidemiology of *Paramphistomum* in ruminants, especially in cows and buffaloes of Khyber Pakhtunkhwa, Pakistan.

2. Material and Methods

Study area and sample collection

Fecal samples were collected from ruminants especially cows and buffaloes in different Districts i.e., Nowshera, Mardan, Swabi, and Charsadda of Khyber Pakhtunkhwa Province, Pakistan, in the months of October 2017 to September 2018 (200 each of cow and buffaloes in each month).

Fecal samples were collected from each area with a total number of 50 fecal samples (5-10 g) from cows and buffaloes month wise in plastic bottles clearly written gender, species, date & place.

Analysis of Fecal Samples

To determine the presence of *Paramphistomum* eggs, all samples were analyzed on the same day by direct microscopic examination and sedimentation methods. Samples which were not observed on the same day of collection were preserved in formalin 10% to prevent eggs development and hatching.

Direct smear method

Few drops of water were added to the fecal samples on microscopic slides. The lighter eggs were allowed to flow away from the heavier debris by tilting the slide. Cover slip was placed on the fluid and the prepared slide was then observed under the microscope (Urquhart et al. 1996).

Sedimentation method

Approximately 2 g of collected sample was mixed in 45 ml of saline water using a spatula and beaker. The fecal suspension was filtered through a filter paper (45 µm), then put in centrifuge tubes as described for centrifuging samples at 1500-2000 rpm. The supernatant was discarded carefully by pipetting (decantation) and the sediment was stained by adding one drop of ethylene blue and then transferred to a microscopic slide. The sample was covered with a coverslip and observed under the microscope (Jorgan 1990).

Based on morphological details, the paramphistomosis eggs were identified by the same technique as described by (Soulsby 1982). The prevalence of Paramphistomosis was noted with the help of formulae described by (Abunna 2012).

3. Results

Paramphistomosis in cows in the selected area of KPK

Overall prevalence in cows

An overall prevalence of 17.5% of Paramphistomosis was found in cows from October 2017 to September 2018.

Month-wise prevalence

For month-wise prevalence, the analysed data showed a highest prevalence of 41% in September followed by 27.5 % in October. The lowest prevalence of 2.5 % of *Paramphistomosis* was recorded in March. Statistical analysis showed no significant difference in the prevalence of *Paramphistomosis* from October 2017 to September 2018, at P<0.05 (Table 1 and Figure 1).

Table 1. Month-wise, season-wise, area-wise, age, and gender-wise prevalence of Paramphistomum in Cows at different Districts of Khyber Pakhtunkhwa.

Factors		Total No. of sample observed	Total No. of sample infected	% age of infection
Month	Oct-17	200	55	27.50%
	Nov-17	200	43	21.50%
	Dec-17	200	39	19.50%
	Jan-18	200	36	18%
	Feb-18	200	51	25.50%
	Mar-18	200	5	2.50%
	Apr-18	200	16	8%
	May-18	200	12	6%
	Jun-18	200	13	6.50%
	Jul-18	200	26	13%
	Aug-18	200	43	21.50%
	Sep-18	200	82	41%
Season	Winter	800	137	17.10%
	Spring	400	26	6.50%
	Summer	800	94	11.75%
	Autumn	400	164	41%
Area	Mardan	600	116	19.30%
	Charsadda	600	83	13.80%
	Swabi	600	115	19.10%
	Nowshera	600	107	17.80%
Age	0-2 years	850	137	16.10%
	>2 years	1550	284	18.30%
Gender	Male	1450	281	19.30%
	Female	950	140	14.70%
Total		2400	421	17.5%

Area-wise prevalence

Area-wise prevalence in cows in various Districts of KPK showed that the infection was highest in Mardan (19.3%) followed by Nowshera (19.1%). The lowest prevalence (13.8%) was observed in Charsadda. Statistical analysis (Student t-test) showed significant differences in all areas (Table 1, Table 2 and Figure 1).

Season-wise prevalence

It is cleared from the results that data collected during autumn season has the highest prevalence 41% compared to winter season which was 17.1% and spring season which was 6.5%. Statistical analysis further clarified our results having significant difference in winter season (P<0.01) compared to summer and spring (Table 1 and Figure 1)

Gender- wise prevalence

Gender-wise analysis of *Paramphistomosis* showed the highest prevalence in males (19.3%) followed by females (14.7%). This indicated that males were more susceptible than females to the disease.

Female animals are found to be more resistance as compared to males. Males and females showed statistical difference at P<0.05 (Table 1 and Figure 1).

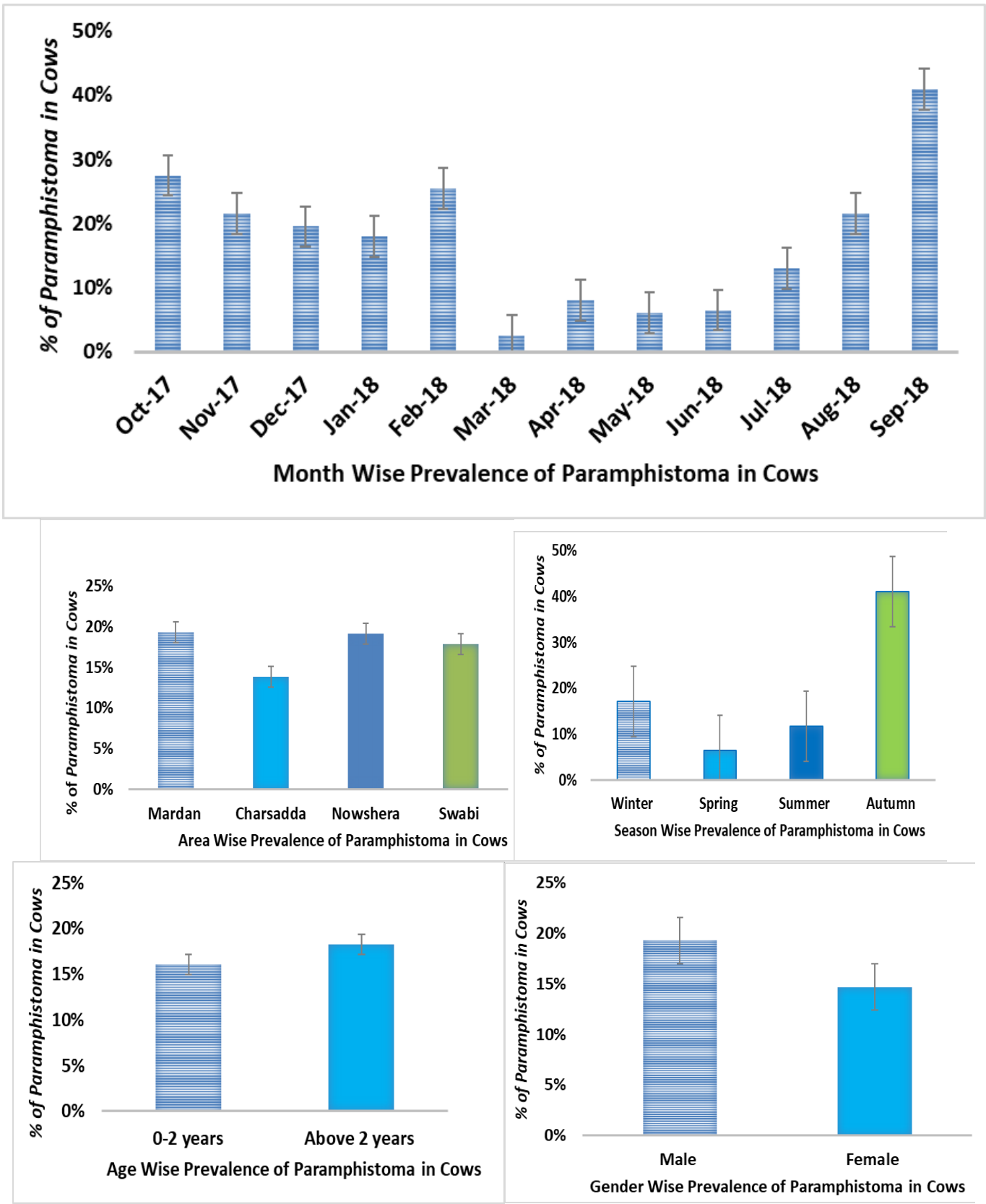

Figure 1. Month Wise, Season wise, Area wise, Age and Gender Wise Prevalence of *Paramphistomum* in Cow in selected areas of Khyber Pakhtunkhwa.

Age-wise prevalence

It was observed that the overall prevalence in adult cows was significantly higher (18.3%) than younger ones (16.1%) (Table 1 and Figure 1).

Table 2. Month wise Prevalence of *Paramphistomum* in Cow in District Mardan, Charsadda, Nowshera and Swabi KPK.

Factors		Mardan		Charsadda		Nowshera		Swabi	
		Inf/total	Prevalence (%)	Inf/Total	Prevalence (%)	Inf/Total	Prevalence (%)	Inf/Total	Prevalence (%)
Month	Oct-17	20/50	40	18/50	36	23/50	46	20/50	40
	Nov-17	19/50	38	5/50	10	20/50	40	8/50	16
	Dec-17	11/50	22	8/50	16	8/50	16	12/50	24
	Jan-18	15/50	30	6/50	12	9/50	18	6/50	12
	Feb-18	7/50	14	2/50	4	4/50	8	2/50	4
	Mar-18	0/50	0	3/50	6	0/50	0	2/50	4
	Apr-18	7/50	14	2/50	4	3/50	6	4/50	8
	May-18	2/50	4	3/50	6	3/50	6	4/50	8
	Jun-18	3/50	6	5/50	10	2/50	4	3/50	6
	Jul-18	4/50	8	7/50	14	9/50	18	6/50	12
	Aug-18	6/50	12	8/50	16	10/50	2	19/50	38
	Sep-18	22/50	44	16/50	32	24/50	48	21/50	42

Overall Prevalence in Buffaloes

Prevalence of *Paramphistomosis* in buffaloes was recorded in selected areas of KPK from October 2017 to September 2018. A total of 409 animal's samples were found infected with overall 17% of infection.

Month-wise prevalence

The prevalence of *Paramphistomosis* showed highest prevalence in September (43.5%) and November (30.5%), followed by October (28.5%). The lowest prevalence has been seen in March (1.5%) (Table 3 and Figure 2).

Statistical analysis showed the highest significant difference in the month of September compared to March 2018 (Table 3 and Figure 2).

Area-wise prevalence

Paramphistomosis prevalence was highest in Swabi (18.8%) while the lowest was seen in Nowshera (13.8%). However, there was no significant difference in prevalence between Swabi and Nowshera (Table 3, Table 4, Figure 2).

Season-wise prevalence

Highest prevalence of *Paramphistomosis* was recorded in autumn (36%) followed by winter (16.7%) and summer (12.8%), while in spring (7%). Statistical analysis also showed significant differences in different seasons (Table 3 and Figure 2).

Gender-wise prevalence

The *Paramphistomosis* prevalence was higher in male (19.5%) as compared to female (13.5%). No significant difference has been seen in male and female buffaloes (Table 3 and Figure 2).

Table 3. Month Wise, Season wise, Area wise, Age and Gender Wise Prevalence of Paramphistomum in Buffalos in selected areas of Khyber Pakhtunkhwa.

Factors		Total No. of sample observed	Total No. of sample infected	% age of infection
Month	Oct-17	200	57	28.50%
	Nov-17	200	61	30.50%
	Dec-17	200	45	22.50%
	Jan-18	200	44	22%
	Feb-18	200	17	8.50%
	Mar-18	200	3	1.50%
	Apr-18	200	16	8%
	May-18	200	10	5%
	Jun-18	200	7	3.50%
	Jul-18	200	25	12.50%
	Aug-18	200	47	23.50%
	Sep-18	200	87	43.50%
Season	Autumn	400	144	36%
	Winter	800	134	16.70%
	Summer	800	103	12.80%
	Spring	400	28	7%
Area	Mardan	600	112	18.60%
	Charsadda	600	101	16.80%
	Swabi	600	83	13.80%
	Nowshera	600	113	18.80%
Age	0-2 years	900	103	11.40%
	>2 years	1500	304	20.20%
Gender	Male	1450	283	19.50%
	Female	950	126	13.20%
Total		2400	409	17%

Table 4. Prevalence of *Paramphistomum* in Buffalos in District Mardan, Charsadda, Nowshera and Swabi KPK.

Factors		Mardan		Charsadda		Nowshera		Swabi	
		Inf/ Total	Prevalence (%)	Inf/ Total	Prevalence (%)	Inf/ Total	Prevalence (%)	Inf/ Total	Prevalence (%)
Month	Oct-17	17/50	34	18/50	36	6/50	12	16/50	32
	Nov-17	20/50	40	14/50	28	8/50	16	19/50	38
	Dec-17	10/50	20	6/50	12	9/50	18	10/50	20
	Jan-18	14/50	28	9/50	18	6/50	12	15/50	30
	Feb-18	7/50	14	4/50	8	1/50	2	5/50	10
	Mar-18	0/50	0	0/50	0	1/50	2	2/50	4
	Apr-18	6/50	12	3/50	6	3/50	6	4/50	8
	May-18	1/50	2	5/50	10	2/50	4	2/50	4
	Jun-18	2/50	4	2/50	4	1/50	2	2/50	4
	Jul-18	4/50	8	7/50	14	8/50	16	6/50	12
	Aug-18	10/50	20	11/50	22	15/50	30	11/50	22
	Sep-18	21/50	42	22/50	44	23	46	21/50	42
Total		112/600	18.6	101/600	16.8	83/60	13.8	113/600	18.8

Age-wise prevalence

A higher prevalence of *Paramphistomosis* was observed in in adult buffaloes above age 2 years (20.2%) than those of young age below 2 years (11.4%). Statistical analysis showed a significant difference at $P<0.05$ between adult and young buffaloes (Table 3, Figure 2).

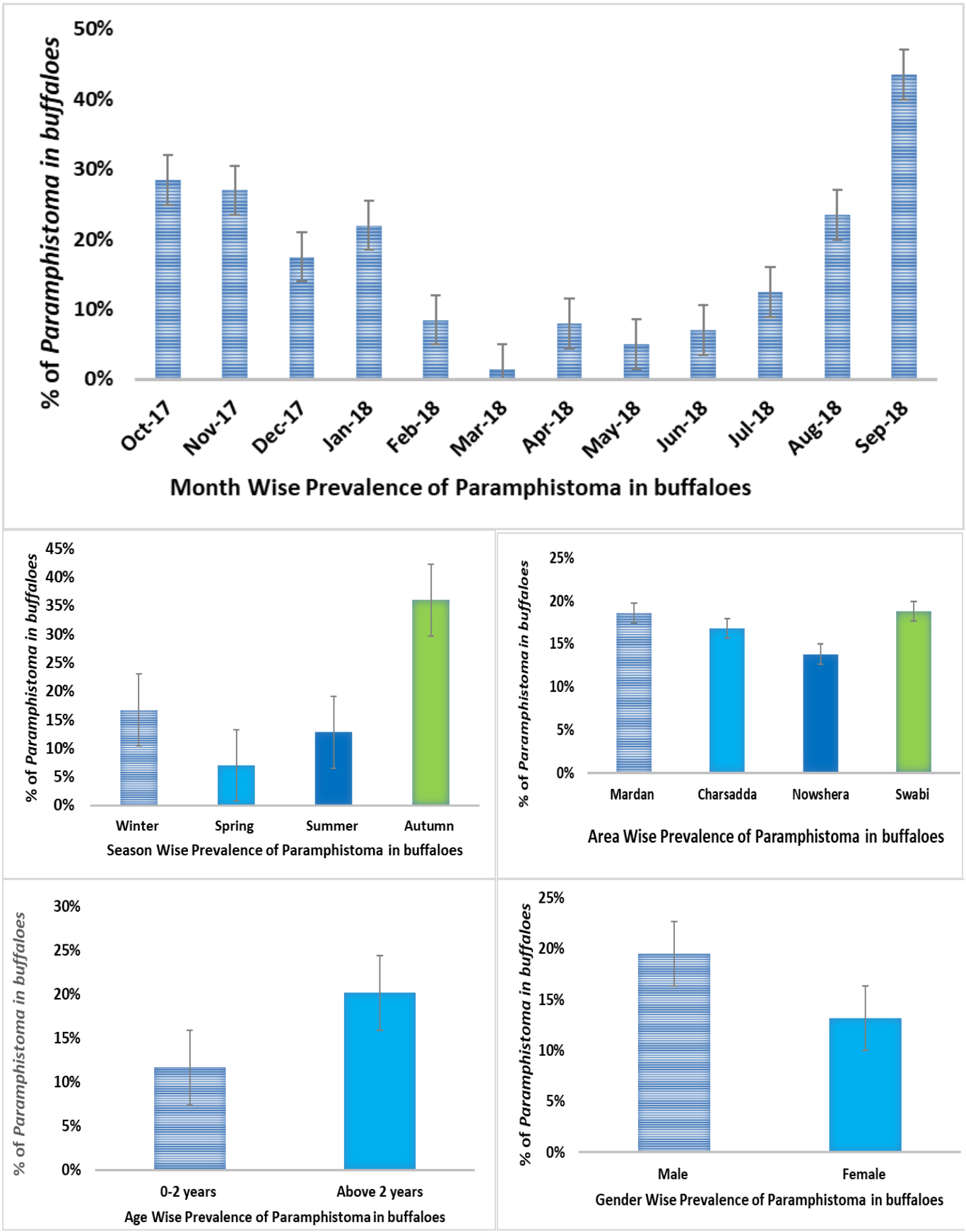

Figure 2. Month-Wise, Season-wise, Area-wise, Age and Gender-Wise Prevalence of *Paramphistomum* in Buffaloes in selected areas of Khyber Pakhtunkhwa.

4. Discussion

In the present study, epidemiological data on *Paramphistomosis* were collected from clinically affected cows and buffaloes from four districts, i.e. "Mardan, Charsadda, Nowshera and Swabi" of the Khyber Pakhtunkhwa province.

Overall, a total of 4800 animals (2400 buffaloes and 2400 cows) were examined from October 2017 to September 2018. Of these, 409 (17%) buffaloes and 421 (17.5%) cows were found positive for *Paramphistomosis*. Similar findings of *Paramphistomum* species were also reported by Kotrla and Kotryl (1982), Chowdhry and Tada (1994), Kassai (1999), Urquhart et al. (1996), Shanila and Hafeez (2005), Dreyfuss et al. (2007) and Khan et al. *(*2008).

Haridy et al. (2006) recorded the prevalence rates of infection were 20% in buffaloes and cattle, similarly 7.3% infection in cattle and 10% in buffaloes in Egypt, and 16.6% in Cattle reported by Jithendran (2000) from India, 25.20% in Buffaloes and 23.8% in Cattle by Juyal et al. (2003) and 5.94% in cattle by Shanila and Hafeez (2005) in India, 13.6% in cattle in Turkey by Sevimli et al. (2005), 7.1 % by Phiri et al. (2006) from Zambian, 28% in cattle from Thailand by Morakot and Sakchai (2006) where as 36% prevalence was observed in cattle from oceanic climatic areas of Lugo (NW Spain) by Diaz et al. (2007)

Seasonal reproduction in *Paramphistomum species* showed striking changes in egg production, with the peak activity during the monsoon and post-monsoon months. Hanna et al. (1988), Gupta and Singh (1990), Chaudhri et al. (1993), Dutta et al. (1995), Georgi et al. (1999), Szmidt et al. (2000), Juyal et al. (2003), Shanila and Hafeez (2005), Keyyu et al. (2005), Pfukeni et at. (2005), Jithendran (2000), Morakot and Sakchai (2006) and Diaz et al. (2007) also recorded similar results.

The highest prevalence in both buffaloes and cows were recorded in September (36.6% in cows and 46.6% in buffaloes) while the lowest (3.3%) in cows during January 2018. Szmidt et al. (2000), Rangel et al. (2003), Juyal et al. (2003), Morakot and Sakchai (2006) and Diaz et al. (2007) also recorded similar results, supporting the present findings.

In the present study, the incidence of *paramphistomosis* was higher in adult animals compared to young. Maqbool et al. (2003), Khan et al. (2006) and Raza et al. (2007) also recorded that younger animals are considered to act as reservoirs of infection than adults, the reason may be that mostly farmers kept the young ones with care and adults mostly grazing freely while Khan et al. (2008) reported totally opposite results, it may be due to climatic conditions and period (year) of study.

It was reported that infection was higher in males (19.5% in buffaloes and 19.3% in cows) compared to female animals (13.2% in buffaloes and 14.7%in cows). In the present study, it was found that infection was slightly lower in females than males, the reason seems to be related to the social practice of keeping females under better management and feeding conditions in comparison to males which are generally grazed freely in grasslands. Khan et al. (2008) and Chaudhri et al. (1993) found the same results.

Sevimli et al. (2005) reported similar findings to the current study in which males with *paramphistomosis* were 3.05 % higher than female animals in comparison to horses Raza et al. (2007) found greater prevalence in males than females in buffaloes. Such findings are in contrast with Szmidt et al. (2000) reports that female cattle in France were substantially more contaminated than male animals due to free grazing by the farmers.

5. Conclusions

The findings of the recorded data indicate that there are no significant differences between the rate of infection of *Paramphistomum* in cows and buffaloes during the study period. The highest prevalence was recorded in autumn and the lowest in spring. It was also observed that old animals are more likely to be infected then young ones due to low immunity at elder age. Female animals are found to be more resistance as compared to males.

Authors' Contributions: RAFIQ, N.: conception and design, acquisition of data, analysis and interpretation of data, and drafting the article; AYAZ, S.: acquisition of data, analysis and interpretation of data, , and drafting the article; NIAZ, S.: acquisition of data, analysis and interpretation of data, drafting the article, and critical review of important intellectual content; KAMAL, M.: acquisition of data, analysis and interpretation of data, drafting the article, and critical review of important intellectual content; KHAN, L.: conception and design, acquisition of data, analysis and interpretation of data, drafting the article, and critical review of important intellectual content. All authors have read and approved the final version of the manuscript.

Conflicts of Interest: The authors declare no conflicts of interest.

Ethics Approval: The present study was approved by the advanced studies and research board, Abdul Wali Khan University Mardan.

Acknowledgments: We are thankful to the Higher Education Commission and Pakistan Science Foundation for their financial support.

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Received: 28 July 2022 | **Accepted:** 18 January 2023 | **Published:** 6 April 2023