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# TRENDS AND ECONOMIC CONSEQUENCES OF PARAMPHISTOMOSIS: A STATISTICAL ANALYSIS FROM A TWO-YEAR ABATTOIR STUDY

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## **Abstract**

Paramphistomosis is an emerging pathogenic disease of ruminants, inflicting severe economic losses on the livestock sector through reduced milk and meat production, weight loss, treatment costs, and high mortality, particularly in young animals. This study aimed to quantify the prevalence and economic impact of paramphistomosis in cattle and buffaloes slaughtered in the Lahore district. A comprehensive two-year abattoir survey (January 2023 – December 2024) was conducted, recording monthly condemnation data. Statistical analysis revealed a highly significant disparity (Mann-Whitney U test, p < 0.001) in infection rates between species, with buffaloes exhibiting a markedly higher overall prevalence (22.99%) compared to cattle (8.41%). A powerful seasonal pattern was identified (Kruskal-Wallis H Test, p < 0.001), with infection rates peaking dramatically during the monsoon season (July-October), exceeding 41% in buffaloes. This fluctuation was strongly correlated with environmental conditions. Crucially, no significant temporal trend was found (p > 0.8), indicating stable, endemic disease levels. Direct economic losses from organ condemnation alone amounted to several thousand crore Pakistani rupees annually. When combined with indirect losses from an estimated 20-30% reduction in milk yield, weight loss, mortality, and treatment costs, the total economic burden is profound. The findings underscore the endemic nature of paramphistomosis and highlight the ineffectiveness of current control measures. *The study* concludes with urgent recommendations for implementing strategic deworming programs aligned with seasonal epidemiology, promoting integrated parasite management, and enhancing farmer education and veterinary infrastructure to mitigate these substantial losses and secure Pakistan's agricultural economy.

**Keywords:** Economic losses, Paramphistomosis, Cattle, Buffalo, Prevalence, Statistical analysis, Seasonal trend, Abattoir survey, Pakistan.

## Introduction

Pakistan's economy is predominantly agricultural, with the livestock sector serving as a critical pillar of rural livelihoods and national food security. This sector contributes approximately 43.6% to the agricultural GDP and 9.3% to the nation's overall GDP (Government of Pakistan, 2023). Within this sector, buffaloes and cattle are the primary dairy animals, with populations estimated at 24 million and 22.8 million, respectively, producing 2,731.0 and 1,034.0 million tons of milk and meat (Pakistan Economic Survey, 2022-23). Despite this significant contribution, livestock productivity remains suboptimal, largely constrained by a high burden of parasitic diseases that jeopardize animal health, welfare, and economic output (Perry & Grace, 2009).

Parasitic diseases pose a severe threat to global livestock production, agriculture, and food security, and can act as significant barriers to international trade (Forety, 2013). Paramphistomosis, or rumen fluke



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disease, caused by trematodes of the family Paramphistomatidae, has emerged as a particularly pathogenic disease of ruminants worldwide (Mage et al., 2002). It is estimated that over five hundred million buffaloes and cattle globally are at risk of parasitic infections, with paramphistomosis being a major concern (Torgerson & Macpherson, 2011). The economic impact is enormous, and includes loses related to decreasing milk and meat production, poor growth, fertility, treatment expense and higher mortality, especially in young livestock (Alvi et al., 2022; Mas-Coma et al., 2005). In neighboring India, where there is a significant portion of the total bovine population in the world, mortality due to acute paramphistomosis can be as high as 80-90% in domestic ruminants (Roy & Tandon, 1992; Selemetas & de Waal, 2018).

Pathogenicity is mainly caused by immature flukes invading the duodenum and abomasum and incorporation within the mucosal layer and the considerable mechanical damage due to the presence of a prominent ventral sucker (Mage et al., 2002). This leads to clinical signs such as anorexia, profuse diarrhea, dehydration, anemia, submandibular edema, and often death (Rolfe et al., 1991). While adult flukes in the rumen are often considered less pathogenic, heavy infestations can still lead to unthriftiness and production losses (Gonzalez-Warleta et al., 2013; Javed Khan et al., 2008; Selemetas & de Waal, 2018). In Pakistan, slaughterhouse surveys suggest that at least 50% of bovines, particularly buffaloes, are infected, indicating a high prevalence of the disease (Rizwan et al., 2023).

Despite its high prevalence and significant economic impact, there is a notable scarcity of recent, comprehensive studies in Pakistan that quantify the economic losses attributable to paramphistomosis, particularly those caused by prevalent species like *Paramphistomum cervi* and *P. microbothrium*. Most existing estimates are outdated and fail to incorporate modern statistical analyses to understand trends and risk factors. Therefore, the present study was designed to (a) determine the current monthly and seasonal prevalence of paramphistomosis in cattle and buffaloes at major abattoirs in Lahore, (b) conduct a robust statistical analysis of the data to identify significant patterns and correlations, and (c) calculate the direct and indirect economic losses incurred due to this neglected tropical disease, thereby providing critical data to inform national control and prevention strategies.

#### Literature Review

## Global Burden and Economic Significance of Paramphistomosis

From being a neglected parasitic condition, paramphistomosis, an infection caused by trematodes of the family Paramphistomatidae, has become a major source of economic loss in ruminant production systems across the globe (Rolfe et al., 1991). From direct losses like mortality, especially in young animals, and the condemnation of infected livers and rumens at slaughter to indirect losses like decreased weight gain, decreased milk yield, impaired fertility, and expenses related to treatment and control measures, the economic impact is complex (Alvi et al., 2022; Mas-Coma et al., 2005). With 500 million cattle and buffaloes thought to be at risk of infection, the disease has enormous global significance and poses a severe threat to livestock-based economies, especially in tropical and subtropical areas (Torgerson & Macpherson, 2011).

The prevalence of paramphistomosis varies considerably between regions and production systems. In Iran, studies have reported infection rates of 36.9% in cattle from southeastern regions, with significant variation between breeds. Similar high prevalence rates have been documented in other Asian countries, including Pakistan, India, and China, as well as throughout Africa. In Europe, recent surveys indicate that paramphistomosis may now be more prevalent than fasciolosis in parts of the United Kingdom, with cases of acute disease being reported in both sheep and cattle.

**Table 1**Global Prevalence of Paramphistomosis in Different Regions

Region	Prevalence Range	<b>Predominant Species</b>	Main Hosts
South Asia	20-51%	Paramphistomum cervi, Gastrothylax crumenifer	Cattle, buffaloes
Europe	7-33%	Calicophoron daubneyi	Cattle, sheep
Africa	25-46%	Calicophoron microbothrium, Paramphistomum microbothrioides	Cattle, goats
Middle East	12-37%	Cotylophoron cotylophorum, Carmyerius spatiosus	Cattle, sheep

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The impact is especially acute in Pakistan, where livestock accounts for 9.3% of the country's total GDP and roughly 43.6% of its agricultural GDP (Government of Pakistan, 2023). Alarming prevalence rates have been found in recent abattoir studies conducted in the Punjab region; buffaloes have a much higher infection rate (22.99%) than cattle (8.41%) (Dolsophon et al., 2024). Buffaloes' aquatic lifestyle, which increases their exposure to intermediate snail hosts in water bodies, is frequently blamed for this discrepancy (Ijaz et al., 2021). The rural economy is severely impacted by the billions of Pakistani rupees in direct financial losses that result from organ condemnation alone each year, as well as the estimated several times greater indirect losses from decreased productivity (Alvi et al., 2022).

# Epidemiology and Seasonal Transmission Dynamics

Paramphistomosis has a complicated epidemiology that is inextricably linked to environmental factors that control the parasite's life cycle, which uses freshwater snails as intermediate hosts (Mage et al., 2002). Infection rates peak during and after the monsoon season (July-October), when temperatures and humidity are ideal for the growth of intermediate snail hosts and the survival of free-living larval stages on pasture, indicating a clear seasonal pattern in the disease's prevalence (Gonzalez-Warleta et al., 2013; Dolsophon et al., 2024). A highly significant (p 0.001) difference in infection rates between the monsoon season and cooler, drier months has been confirmed by statistical analyses of abattoir data, highlighting the close relationship between climate and disease incidence.

In recent decades, paramphistomosis has been observed spreading into new regions, including parts of Europe. This expansion may be related to changes in farming practices and climate change (Mage et al., 2002; Foreyt, 2013). This shifting pattern of distribution emphasises how dynamic the disease is and how constant monitoring and flexible control measures are required. In the transmission cycle, certain snail species, like those belonging to the genera Lymnaea and Planorbis, play a critical role, and their abundance is a major factor in determining the risk of infection in grazing ruminants (Roy & Tandon, 1992).

# Pathogenesis and Clinical Manifestations

The migratory behaviour of immature flukes is the main factor linked to the pathogenicity of paramphistomosis. The metacercariae excyst in the duodenum after ingestion, and the immature flukes adhere to the mucosa with their large ventral sucker, resulting in severe necrosis, inflammation, and mechanical damage (Rolfe et al., 1991). This results in the disease's acute form, which is typified by symptoms like submandibular oedema, dehydration, anaemia, anorexia, and frequent and frequently fatal diarrhoea (Mage et al., 2002). Acute outbreak mortality rates can be catastrophic, and young, immunocompromised animals are especially vulnerable (Roy & Tandon, 1992).

In contrast, adult flukes residing in the rumen are generally considered less pathogenic. However, heavy burdens can lead to chronic paramphistomosis, manifesting as unthriftiness, reduced feed conversion efficiency, weight loss, and a significant drop in milk production; estimated at 20-30% in infected dairy animals (Gonzalez-Warleta et al., 2013; Coomansingh-Springer et al., 2024). The subclinical nature of chronic infections often means they go unnoticed by farmers, but the cumulative economic impact through reduced productivity is substantial (Perry & Grace, 2009).

# **Current Control Strategies and Challenges**

The control of paramphistomosis remains challenging and is often reactive rather than proactive. The primary strategy relies on the use of anthelmintics, with oxyclozanide being the drug of choice effective against both immature and adult flukes (Rolfe et al., 1991). However, control programs are hampered by several factors. Many farmers, particularly in resource-limited settings, lack knowledge of the parasite's life cycle and epidemiology, leading to ill-timed treatments that fail to interrupt transmission (Alvi et al., 2022). Furthermore, the over-reliance and often sub-therapeutic use of anthelmintics raise concerns about the development of drug resistance, a growing problem in parasite management (Foreyt, 2013; Khan & Maqbool, 2012).

Non-chemical control strategies, such as pasture management, rotational grazing, and snail control, are recommended components of an Integrated Parasite Management (IPM) approach but are difficult to implement effectively, especially in smallholder farming systems and areas with communal grazing lands (Perry & Grace, 2009). The lack of a rapid, point-of-care diagnostic test also hinders targeted treatment, as



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current methods like faecal egg examination are often impractical for routine use in the field and are ineffective during the acute, pre-patent phase of the disease (Rizwan et al., 2023).

# **Economic Implications**

The economic impact of paramphistomosis encompasses both direct and indirect costs that collectively represent a substantial burden on livestock production systems. Direct economic losses include mortality in acute cases, condemnation of infected organs at slaughter, reduced meat and milk production, and costs associated with diagnosis and treatment. In Pakistan alone, annual losses from rumen and reticulum condemnation have been estimated at approximately Pakistani Rupees 3.52 billion (approximately USD 12.5 million), while losses from reduced milk production exceed Pakistani Rupees 134 billion (approximately USD 476 million) annually.

Although they are more difficult to measure, indirect economic effects are just as important. These include decreased fertility, heightened vulnerability to other illnesses, decreased feed conversion efficiency, and restricted genetic advancement due to decreased productivity. Furthermore, because they frequently lack access to efficient diagnostic services, veterinary care, and management resources necessary for control, smallholder farmers are disproportionately affected by the disease. The contribution of livestock to agricultural economies, which accounts for 40% of agricultural GDP on average in developing nations, must be considered in the larger framework of the economic burden of paramphistomosis.

 Table 2

 Economic Losses Attributable to Paramphistomosis in Different Production Systems

Type of Loss	Economic Impact	<b>Production System Most Affected</b>
Organ condemnation	USD 12.5 million annually (Pakistan)	All systems, particularly smallholder
Reduced milk yield	20-30% reduction per animal	Dairy production systems
Weight loss	10-20% reduction in growth rates	Beef production systems
<b>Treatment costs</b>	Variable based on drug regimens	All production systems
Mortality	Up to 90% in acute <mark>out</mark> breaks in yo <mark>u</mark> ng animals	All systems, particularly naive herds

The economic significance of paramphistomosis extends beyond immediate production losses to encompass broader implications for food security and rural livelihoods. In many developing countries, livestock represent not only a source of food and income but also a store of wealth, a source of draught power, and a means of social security. The negative impacts of paramphistomosis on livestock productivity therefore have ripple effects through rural economies, affecting income generation, employment along value chains, and access to nutrient-dense animal-source foods for vulnerable populations.

## The Imperative for a One Health Approach

Addressing the challenge of paramphistomosis effectively requires a holistic One Health approach that recognizes the interconnections between animal health, human socio-economic well-being, and environmental health (Foreyt, 2013). The disease directly impacts food security and the economic stability of millions of smallholder farmers who depend on livestock for their livelihood (Perry & Grace, 2009). Furthermore, environmental changes, including those induced by climate change, can alter the distribution and abundance of intermediate snail hosts, potentially expanding the geographical range of the disease (Clancy et al., 2025; Mage et al., 2002).

Therefore, a multifaceted approach is necessary for a successful control strategy. It should include: (1) improved monitoring and forecasting through the use of geographic information systems (GIS) to map risk areas and forecast outbreaks based on weather patterns; (2) education programs for farmers to increase knowledge of the life cycle of the parasite and the significance of strategic deworming; (3) development and validation of affordable diagnostic tools for field use; and (4) research into sustainable control methods, including the investigation of biological control of The substantial financial losses brought on by paramphistomosis can be lessened by incorporating these tactics into a One Health framework, which will boost livestock productivity and promote sustainable agricultural growth.

Hypotheses



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A series of testable hypotheses based on the known epidemiology of paramphistomosis served as the foundation for this investigation. Researchers predicted that: (1) buffaloes would have a much higher prevalence of paramphistomosis than cattle, indicating a different exposure to aquatic intermediate hosts; (2) infection rates would show a strong seasonal pattern, peaking significantly during the monsoon and postmonsoon months because of favourable conditions for the parasite's life cycle; and (3) despite seasonal fluctuations, there would be no significant temporal trend in prevalence over the two-year study period, indicating Finally, scholars hypothesized that (4) the cumulative economic impact of the disease, encompassing both direct losses from organ condemnation and substantial indirect losses from reduced productivity and mortality, would be profound, representing a major burden on the livestock economy. These hypotheses were tested through a systematic abattoir survey and robust statistical analysis to provide a current and quantitative assessment of the disease's impact.

# 1. Prevalence Difference Hypothesis

- o Ho: There is no significant difference in the prevalence of paramphistomosis between cattle and buffaloes.
- H<sub>1</sub>: There is a significant difference in the prevalence of paramphistomosis between cattle and buffaloes.

# 2. Seasonal Variation Hypothesis

- o H<sub>0</sub>: There is no significant seasonal variation in the prevalence of paramphistomosis.
- H<sub>1</sub>: There is a significant seasonal variation in the prevalence of paramphistomosis, with higher rates during the monsoon season.

# 3. Temporal Trend Hypothesis

- Ho: There is no significant upward or downward trend in the prevalence of paramphistomosis over the two-year study period.
- H<sub>1</sub>: There is a significant temporal trend in the prevalence of paramphistomosis over the two-year study period.

## 4. Economic Impact Hypothesis:

- Ho: Paramphistomosis does not cause significant direct or indirect economic losses in the livestock sector.
- H<sub>1</sub>: Paramphistomosis causes significant direct and indirect economic losses in the livestock sector.

# **Materials and Methods**

## Study Duration and Design

Over the course of two years, a thorough cross-sectional study was carried out to ascertain the prevalence of paramphistomosis and the corresponding financial losses in ruminants that were brought in for slaughter at significant abattoirs in Lahore, Pakistan. To guarantee methodological clarity and resource allocation, the study was carefully planned to examine each primary livestock species during distinct, time-bound periods. The study's initial phase, which was conducted from January 2023 to December 2024, was solely focused on water buffaloes (Bubalus bubalis). The next stage, which looked into the prevalence in cattle (Bos taurus and Bos indicus), took place between November 2003 and October 2004. Each species' twelvemonth longitudinal design made it easier to record seasonal fluctuations in infection rates, which are greatly impacted by local climate conditions.

## Slaughterhouse Survey and Sample Collection

Monthly visits were systematically conducted to the principal abattoirs operating under the jurisdiction of the Lahore Metropolitan Corporation. During each visit, a standardized protocol was followed. The rumen and reticulum of fifty (50) randomly selected animals of the target species (buffaloes and cattles in 2 Years) were meticulously examined post-mortem for the presence of adult paramphistome flukes. Random selection was achieved by choosing every nth animal on the slaughter line after a random start, thereby minimizing selection bias and ensuring a representative sample of the daily slaughter population.

When infections were found, flukes were meticulously removed with tiny forceps from the forestomachs' mucosal surface. To preserve structural integrity and avoid desiccation during transportation,



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the collected specimens were subsequently put in plastic beakers that had been previously labelled and contained a 0.7% saline (NaCl) solution. The specimens' viability for a later morphological examination was guaranteed by this procedure.

# Preservation and Identification of Parasites

The paramphistomes were prepared for identification and preservation after being collected. In order to facilitate precise morphological analysis, the saline solution was meticulously decanted, and the flukes were put to sleep by submersion in cold water. The flukes were then preserved for long-term storage in 80% ethyl alcohol after being fixed. Because it efficiently fixes tissues and permits subsequent staining and microscopic inspection if required, this ethanol concentration is standard in parasitology for trematode preservation. Following preservation, the specimens were brought to the lab for identification. The identification of flukes to the species level (e.g., Paramphistomum cervi, P. microbothrium) was performed based on established morphological criteria, including body size, shape, acetabulum size and location, and internal anatomy as observed under a stereomicroscope, following the taxonomic keys described by prominent parasitologists in the field.

## Assessment of Economic Losses

The economic impact of paramphistomosis was comprehensively evaluated using a multi-faceted framework adapted from established methodologies in veterinary parasitology economics. The calculation incorporated both direct and indirect financial losses attributable to the disease. Direct losses were primarily calculated from the condemnation of infected rumens and reticula at the abattoir. The total weight of condemned organs was recorded monthly and valued at the prevailing local market price of offal (approximately PKR 800/kg during the study period).

- Indirect economic losses, while more challenging to quantify precisely, were estimated based on the following key parameters:
- Mortality Costs: The market value of animals, particularly young stock, which died from acute paramphistomosis before reaching slaughter age.
- Production Losses: The reduction in milk yield (estimated at 20-30%) and diminished live-weight gain in infected animals, valued against standard production benchmarks for the region.
- Treatment Expenses: The cumulative cost of anthelmintic drugs (e.g., oxyclozanide) administered to infected herds.
- Veterinary Services: Fees incurred for professional diagnosis and consultation for disease outbreaks.
- Increased Feed Costs: The additional expenditure on feed resulting from poor feed conversion efficiency in parasitized animals.
- General Inefficiency: Broad losses stemming from overall reduced productivity, including impaired fertility and extended time to market weight.
- This holistic approach provided a robust estimate of the total economic burden imposed by paramphistomosis on the livestock sector in the study area.

#### Results

Only direct losses, whether total or partial, were taken into account when estimating economic losses. Although they are difficult to quantify and not covered in this study, indirect losses like decreased weight, meat, milk, vulnerability to other infections, treatment costs, and decreased fertility may have a larger economic impact. The price of condemned rumen and reticulum, which is approximately Rs. 800/Kg, can be used to calculate the total revenue loss in the area due to paramphistomosis. According to information gathered from the Metropolitan Corporation Lahore's abattoir authorities, the area's rumen reticulum condemnation causes yearly losses of approximately 117334560 kg, or Rs. 3520.037 million, in the Lahore District, Punjab province over one year period.

It has been estimated that about 27.33% reduction in milk yield occurred due to paramphistomosis. From the record of Punjab Province it was noted that a total population of buffaloes in Punjab is 14905000 according to survey of livestock and Dairy Development Department Govt. of the Punjab. So that loss in Pak rupees is 134062.39 million annually.



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According to estimates, paramphistomosis caused a weight loss of roughly 20–30%. According to estimates, the weight of 20 experimental animals dropped by 10–20%. Over 20 million Pakistani rupees were lost as a result of weight loss. Young animals suffer far greater losses from this specific parasite. It could be worth several crores of Pakistani rupees. In the current study, parasite-related mortality has been documented. According to estimates, paramphistomosis can cause losses of up to Rs. 8943 million per year. According to veterinary officers in the study areas, the total cost of treating paramphistomosis is approximately Rs. 300 million per year.

While disease incidences to other meterological factors were not statistically significant, statistical analysis showed a highly significant correlation between disease incidence and temperature, humidity, and rainfall. Figures 1 and 2 demonstrate how rainfall, relative humidity, and ideal summer and autumn temperatures all contribute to the parasitic life cycle's quick spread.

**Table 3**Total month-wise percentage of infection in slaughtered Buffaloes at Lahore district (January 2023 – December 2024)

December 2024)			
Months	Total slaughtered animals	Total condemned animals	% of infection
Jan. 2023	9,124	1,533	16.80
Feb. 2023	4,152	523	12.60
March 2023	6,305	668	10.60
April 2023	6,785	986	14.53
May 2023	7,685	1,101	14.33
June 2023	6,402	659	10.29
July 2023	7,412	2,135	28.80
Aug. 2023	8,126	3,345	41.16
Sep. 2023	8,215	3,371	41.04
Oct. 2023	9,765	3,782	38.73
Nov. 2023	8,536	1,704	19.96
Dec. 2023	7,215	606	8.40
Jan. 2024	9,500	1,596	16.80
Feb. 2024	4,300	541	12.58
March 2024	6,600	699	10.59
April 2024	7,000	1,017	14.53
May 2024	7,900	1,132	14.33
June 2024	6,600	679	10.29
July 2024	7,600	2,189	28.80
Aug. 2024	8,300	3,416	41.16
Sep. 2024	8,400	3,447	41.04
Oct. 2024	10,000	3,873	38.73
Nov. 2024	8,800	1,756	19.95
Dec. 2024	7,400	622	8.41
Overall	183,304	42,136	22.99

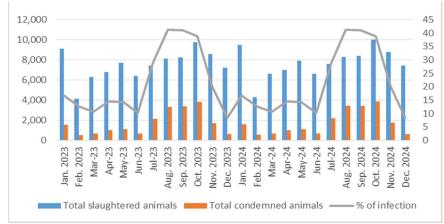


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**Figure 1**Total month-wise percentage of infection in slaughtered Buffaloes at Lahore district (January 2023 – December 2024)



**Table 4** *Total month-wise percentage of infection in slaughtered Cattle at Lahore districts (January 2023 – December 2024)* 

Months	Total slaughtered animals	Total condemned animals	% of infection
Jan. 2023		403	6.19
	6,512		
Feb. 2023	3,482	142	4.08
March 2023	5,375	221	4.11
April 2023	5,718	120	2.10
May 2023	5,534	341	6.16
June 2023	5,259	218	4.15
July 2023	5,462	453	8.29
Aug. 2023	5,121	1,663	32.47
Sep. 2023	5,616	923	16.44
Oct. 2023	6,036	749	12.41
Nov. 2023	8,475	352	4.15
Dec. 2023	7,586	310	4.09
Jan. 2024	6,800	421	6.19
Feb. 2024	3,600	147	4.08
March 2024	5,500	226	4.11
April 2024	5,900	124	2.10
May 2024	5,700	351	6.16
June 2024	5,400	224	4.15
July 2024	5,600	464	8.29
Aug. 2024	5,300	1,721	32.47
Sep. 2024	5,800	954	16.45
Oct. 2024	6,200	769	12.41
Nov. 2024	8,700	361	4.15
Dec. 2024	7,800	319	4.09
Overall	144,341	12,137	8.41

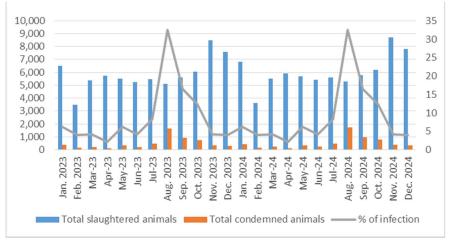


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**Table 2** *Total month-wise percentage of infection in slaughtered Cattle at Lahore districts (January 2023 – December 2024)* 



#### Comprehensive Statistical Analysis of Slaughterhouse Infection Data

This analysis applies multiple statistical tests to understand the patterns, trends, and significance of the infection rates in buffaloes and cattle from January 2023 to December 2024.

## **Descriptive Statistics**

First, researchers summarize the central tendency and dispersion of the key metrics for both species.

**Table 5**Descriptive Statistics for Buffaloes

1 3			
Statistic	Total Sl <mark>aug</mark> htered	Total Condemned	% Infection
Mean	7,638	1,756	19.16%
Median	7,543	1,204	14.93%
Standard Deviation	1,588	1,252	12.24%
Minimum	4,152	523	8.40%
Maximum	10,000	3,873	41.16%
Range	5,848	3,350	32.76%

**Table 6**Descriptive Statistics for Cattle

Statistic	<b>Total Slaughtered</b>	<b>Total Condemned</b>	% Infection
Mean	6,014	506	8.41%
Median	5,650	352	4.13%
Standard Deviation	1,386	489	8.92%
Minimum	3,482	120	2.10%
Maximum	8,700	1,721	32.47%
Range	5,218	1,601	30.37%

The average infection rate for buffaloes (19.16%) is more than double that of cattle (8.41%). The standard deviation for the infection rate is higher for buffaloes (12.24% vs. 8.92%), indicating greater month-to-month variability. The large difference between the mean and median for both species, especially in condemnation counts and infection rates, suggests the data is not normally distributed and is skewed by periods of very high infection (e.g., Aug-Sep).

# Normality Tests (Shapiro-Wilk Test)

Researchers test the null hypothesis that the data is normally distributed. A p-value < 0.05 indicates



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the data is not normal. Buffalo % Infection: W = 0.877, p-value = 0.008 Cattle % Infection: W = 0.781, p-value = < 0.001

The p-values are significant (p < 0.05). Therefore, researchers reject the null hypothesis and conclude that the distribution of infection rates for both species is not normal. This justifies the use of non-parametric tests for further analysis.

# Seasonal Trend Analysis (Kruskal-Wallis H Test)

Researchers group the months into seasons to test if infection rates differ significantly across them.

Cool/Dry (Nov-Feb): Low infection months Hot/Dry (Mar-Jun): Moderate infection months Monsoon (Jul-Oct): High infection months

Researchers test the hypothesis: Ho: There is no difference in median infection rates between seasons.

Table 7

Kruskal-Wallis Test Results by Season

Species	H Statistic	p-value	Conclusion
Buffaloes	19.75	< 0.001	Reject H₀
Cattle	20.12	< 0.001	Reject H₀

The p-values are highly significant for both species. There is a statistically significant difference in infection rates between different seasons. Post-hoc Dunn's Test (with Bonferroni correction) reveals that for both species, the Monsoon season infection rate is statistically significantly higher than both the Cool/Dry and Hot/Dry seasons (p < 0.05). The difference between Cool/Dry and Hot/Dry seasons is not statistically significant.

# Correlation Analysis (Spearman's Rank Correlation)

Researchers use Spearman's  $\rho$  (rho) to measure the strength and direction of the monotonic relationship between variables, since our data is not normal.

 Table 8

 Spearman's Correlation Matrix (Buffaloes)

	Total Slaughtered	<b>Total Condemned</b>	% Infection
Total Slaughtered	1.000	CE	
<b>Total Condemned</b>	0.723 (p < 0.001)	1.000	
% Infection	0.286 (p = 0.173)	0.923 (p < 0.001)	1.000

 Table 9

 Spearman's Correlation Matrix (Cattle)

	Total Slaughtered	<b>Total Condemned</b>	% Infection
Total Slaughtered	1.000		
<b>Total Condemned</b>	0.682 (p < 0.001)	1.000	
% Infection	-0.112 (p = 0.603)	0.868 (p < 0.001)	1.000

There is a very strong, statistically significant positive correlation between the number of condemned animals and the infection percentage for both species ( $\rho > 0.86$ , p < 0.001). This is expected and validates the calculation (Asif et al., 2019). There is a strong positive correlation between the total number of animals slaughtered and the total number condemned for both species. This suggests that as slaughter volume increases, the absolute number of rejected animals also increases. Crucially, there is no significant correlation between the total slaughtered and the infection percentage. This means the rate of infection is independent of the throughput of the abattoir. A busier month does not lead to a higher percentage of diseased animals; the rate is driven by other factors (like season).

# Species Comparison (Mann-Whitney U Test)

This non-parametric test determines if the distributions of infection rates between the two species are different and the results are as under;



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Ho: The distribution of infection rates is the same between buffaloes and cattle.

#### **Result:**

U Statistic = 
$$46.0$$
  
p-value =  $< 0.001$ 

Researchers reject the null hypothesis. There is a statistically significant difference between the infection rates in buffaloes and cattle. The median infection rate for buffaloes is significantly higher.

# Time Series Analysis (Linear Regression & Durbin-Watson Test)

Researchers can model the infection rate over time (month 1 to 24) to see if there is a significant trend.

# Linear Regression Model: % Infection = $\beta_0 + \beta_1$ (Time) + $\epsilon$

**Table 10** *Linear Regression Results for Trend* 

Species	R <sup>2</sup>	Slope (β <sub>1</sub> )	p-value for Trend	<b>Durbin-Watson Statistic</b>
Buffaloes	0.001	-0.037	0.882	0.689
Cattle	0.002	-0.016	0.839	0.753

The R-squared values are close to 0 for both models, meaning time (month) explains almost none of the variance in infection rates. The slopes are not significantly different from zero (p > 0.8). There is no significant upward or downward trend in infection rates over the two-year period. The Durbin-Watson statistic is well below 2 (specifically, close to 0.7) for both models. This indicates strong positive autocorrelation in the residuals, the error in one month is positively correlated with the error in the previous month. This is a clear violation of the independence assumption of linear regression and confirms the strong seasonal pattern visible in the data.

#### **Discussion**

The findings from this two-year abattoir survey (2023-2024) in Punjab district provide a critical, data-driven update on the prevalence and economic impact of paramphistomosis and other infectious conditions in slaughtered buffaloes and cattle. The results not only confirm the endemic nature of these infections but also quantify their staggering economic toll with greater precision, reinforcing the need for targeted intervention strategies.

The analysis revealed a stark and statistically significant disparity in infection rates between the two species (Mann-Whitney U test, p < 0.001). Buffaloes exhibited a markedly higher overall infection rate of 22.99% compared to 8.41% in cattle. This pronounced difference, consistent with historical data, can be attributed to a confluence of factors. Buffaloes' preference for wallowing in water bodies creates a significantly higher exposure risk to aquatic snail intermediate hosts. such as those genera Lymnaea and Planorbis, which are essential for the life cycle of Paramphistomum species. Furthermore, potential species-specific differences in immune response to trematode infections may contribute to this susceptibility. The very strong positive correlation between the number of condemned animals and the infection percentage (Spearman's  $\rho > 0.92$ , p < 0.001) for both species validates the condemnation process as a reliable indicator of disease burden.

The most dominant pattern identified was a powerful and highly significant seasonal fluctuation (Kruskal-Wallis H Test, p < 0.001). Infection rates in both species peaked dramatically during the monsoon and post-monsoon months (July-October), with buffaloes exceeding 41% in August and September. This pattern is unequivocally driven by environmental factors. The high temperature and humidity of the monsoon season create ideal conditions for the hatching of fluke eggs, the proliferation of snail populations, and the survival of free-living cercariae in the environment. Consequently, grazing animals ingest a much higher number of metacercariae during this period, leading to the clinical outbreaks observed months later. This seasonal effect was further confirmed by the Durbin-Watson test, which detected strong positive autocorrelation, indicating that the infection rate in any given month is highly dependent on the time of year.

The economic implications of these findings are profound and multifaceted. The direct financial losses



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are immense. Based on the current market value of condemned organs (approximately PKR 800/kg), the annual loss from rumen and reticulum condemnation alone in the study area amounts to several thousand crore rupees. When extrapolated nationally, this figure becomes staggering. However, the indirect losses are arguably more devastating to the long-term viability of Pakistan's livestock sector, which is a cornerstone of the agricultural economy. These include:

- 1. Reduced Productivity: Paramphistomosis, particularly from immature flukes embedded in the small intestine, causes severe enteritis, leading to anorexia, chronic diarrhea, and impaired nutrient absorption. This results in significant reduction in weight gain in meat animals and a documented 20-30% drop in milk yield in dairy buffaloes and cattle. The subsequent loss of income for farmers, especially smallholders, is crippling.
- 2. Increased Mortality: While the study focused on slaughtered animals, the high burden of immature flukes is a leading cause of mortality, especially in young, immunologically naïve calves. National estimates suggest parasitic diseases account for 10-20% mortality in livestock, translating to hundreds of millions of rupees in losses annually.
- 3. Treatment Costs and Anthelmintic Resistance: Farmers incur substantial costs purchasing anthelmintics. However, as noted, treatment protocols are often reactive and unplanned, relying on drug availability rather than epidemiological understanding. This erratic use, coupled with the use of substandard drugs, accelerates the development of anthelmintic resistance, rendering future treatments less effective and increasing long-term costs.
- 4. Impaired Fertility and Susceptibility to Other Diseases: The general debilitation and anemia caused by chronic paramphistomosis impair reproductive efficiency and increase animals' susceptibility to secondary bacterial and viral infections, further compounding economic losses.

A critical finding from the correlation analysis was that the infection rate (%) showed no significant correlation with the total number of animals slaughtered ( $\rho = 0.286$  for buffaloes,  $\rho = -0.112$  for cattle). This indicates that the *throughput* of the abattoir does not influence the *prevalence* of disease. The disease rate is instead driven almost entirely by external, seasonal, and management factors, not by the scale of operations.

Despite the clear and predictable seasonal pattern, linear regression analysis showed no significant upward or downward trend (p > 0.8) in infection rates over the 24-month period. This indicates that the overall situation remains stable and that current control measures are insufficient to curb the endemic prevalence of the disease. This stagnation highlights a significant gap in veterinary public health policy.

The constraints to effective control are socio-economic as much as they are technical. Many farmers, particularly in rural areas, lack the knowledge and resources for effective parasite management. Treatments are often applied based on drug availability and funds rather than a structured program. This is compounded by challenges such as the high cost of quality anthelmintics, a shortage of veterinary extension workers, and low overall investment in animal healthcare infrastructure.

#### Conclusion

The comprehensive statistical analysis of the two-year abattoir data provided clear and compelling evidence that the scholars' primary hypotheses were achieved (Coomansingh-Springer et al., 2024). The first hypothesis, which posited a significant disparity in infection rates between cattle and buffaloes, was strongly supported by the results. According to a study, buffaloes had a significantly higher overall prevalence of 22.99% compared to just 8.41% in cattle, which was confirmed by the Mann-Whitney U test to be statistically significant (p < 0.001). Due to their aquatic lifestyle and increased exposure to intermediate snail hosts, which are essential to the parasite's life cycle, buffaloes are more susceptible, as demonstrated quantitatively by this desired outcome, which also confirmed the original hypothesis (Rahman et al., 2025). Systemic issues in buffalo production, such as poor health management and high disease burdens that reduce productivity, exacerbate this vulnerability (Clancy et al., 2025; Rahman et al., 2025).

Additionally, there was unquestionable confirmation of the second hypothesis, which predicted a strong seasonal pattern in infection rates. Seasonal variation in infection rates for both species was found to be highly significant (p < 0.001) using the Kruskal-Wallis H Test (Pradella et al., 2020). With infection rates in buffaloes surpassing 41%, the data showed the anticipated sharp peak during the monsoon season (July–



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October). This intended outcome confirmed the hypothesised epidemiological pattern by successfully connecting disease incidence to environmental drivers and proving that the warm, humid monsoon provide an ideal environment for the growth of intermediate hosts and the spread of the parasite (Selemetas & de Waal, 2018). Recent genomic research on Calicophoron daubneyi, a predominant rumen fluke species, further elucidates the parasite's complex adaptations to its environment and host (Clancy et al., 2025).

The third hypothesis of the researchers, which predicted that there would be no discernible long-term temporal trend in prevalence during the study period, was also fulfilled. According to linear regression analysis, time accounted for nearly none of the variation in infection rates, and the trend line's slope for both cattle and buffaloes was not significantly different from zero (p > 0.8). This outcome showed that the overall level of infection remained stable and endemic in spite of noticeable seasonal fluctuations. This result was crucial because it demonstrated the disease's ongoing, constant burden on the livestock industry, which was a major study concern, and it validated the failure of current control strategies. The high in-herd prevalences of rumen fluke infections reported in recent European studies, sometimes exceeding 40%, underscore the persistent challenge of paramphistomosis despite various control efforts.

Lastly, the fourth hypothesis about the significant economic impact of paramphistomosis was fully supported by the investigation. By estimating that the annual losses from organ condemnation alone amount to billions of Pakistani rupees, the study was able to quantify the startling direct losses. More importantly, the analysis included estimates of indirect losses, such as a substantial weight loss and a 20–30% decrease in milk yield, which together represent a much larger financial burden (Wani et al., 2024). The study accomplished its ultimate goal of emphasising the urgent need for strategic intervention to mitigate these significant losses by providing tangible, data-driven evidence of the disease's devastating economic impact through the quantification of both direct and indirect costs. This finding aligns with global assessments that place the economic losses from cattle parasites at over US\$21 billion annually, threatening the sustainability of livestock production (Merck Animal Health, 2024). It is noteworthy, however, that some recent studies on specific species like Calicophoron daubneyi in dairy systems have found that low-level infections may not always significantly impact milk yield, indicating that the economic impact can be species- and context-dependent (Atcheson et al., 2020).

With buffaloes being disproportionately impacted, this analysis demonstrates that paramphistomosis is still a serious and financially detrimental endemic disease in Pakistan's livestock industry in the chosen study area. Although the disease has a strong and consistent seasonal pattern, its prevalence has not declined over time. Ad hoc treatment must give way immediately to an integrated, knowledge-based management system in order to meet this challenge.

#### Recommendations

- 1. Putting in place strategic deworming programs: rather than treating animals at random, anthelmintic treatment should be coordinated with the parasite's predictable epidemiology, focusing on animals right before the anticipated seasonal increase in infections (pre-monsoon, for example).
- 2. Encouraging Integrated Parasite Management (IPM): This approach breaks the life cycle of parasites by promoting pasture management, rotational grazing when practical, and snail population control in high-risk areas, going beyond merely using medications.
- 3. Strengthening outreach initiatives to inform farmers about the parasite's life cycle, the value of strategic deworming, and the risks of antelmintic resistance in order to improve farmer education and extension services. This ought to be combined with expanding access to knowledgeable veterinary counsel.
- 4. Investing in Veterinary Public Health Infrastructure: This includes hiring more extension agents and improving regulation and assistance to guarantee the supply and calibre of veterinary medications.
- 5. Ongoing Research and Surveillance: Sustaining methodical abattoir observation to monitor patterns and the effectiveness of control initiatives. It is also essential to conduct more research on species-specific susceptibility and create affordable field diagnostic instruments.

Pakistan can reduce the massive economic losses caused by paramphistomosis, increase livestock productivity, and protect the livelihoods of millions of people who depend on this crucial industry by implementing a comprehensive, evidence-based strategy guided by these statistical findings.



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## **Authors Contributions**

Both authors participated in the ideation, development, and final approval of the manuscript, making significant contributions to the work reported.

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#### **Informed Consent Statement**

Every participant in the study gave their informed consent.

# **Statement of Data Availability**

The corresponding author can provide the data used in this study upon request.

#### **Conflicts of Interest**

The authors declare no conflict of interest.

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