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TABLE OF CONTENTS

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The Role of Zoos in Biodiversity Conservation

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The term zoo refers to a variety of institutions that house wild or exotic animals. Zoos can develop effective conservation strategies for endangered species, conduct scientific study to improve animal husbandry, impacts of the living collection, and employ educational initiatives to promote human interaction, development, and behavioral change due to their wide global reach. The commitment to biodiversity conservation guides day-to-day operations at zoos globally.

Zoos, as organizations mostly deal with different aspects of conservation like practice, research and advocacy. Conservation practices include species reintroduction initiatives, captive breeding, species survival strategies, and the utilization of zoo revenue for conservation programs in the wild. Zoos offer captive breeding programs for endangered species to regulate genetic diversity and maintain their population. The key aspect in conservation is advocacy which contributes to public engagement, supports stewardship, raises awareness and initiates fundraising programs, ultimately leading to protective environment for all kinds of species.

Zoos provide knowledge on animal conservation, care, and confinement by conducting research and developing methodologies in wildlife biology, animal behavior, health, and welfare.They also promote educational and research initiatives in collaboration with scientists interested in animal conservation and preservation and ecological diversity. They inspire visitors to develop a relationship with wildlife by allowing them to observe live animals. Public engagement can ensure public awareness by communicating importance of biodiversity and advocates wildlife conservation.

Zoos serve an important role in restoring population of threatened and endangered species, protecting their habitats, maintaining genetic diversity, and developing new strategies for species conservation. Animal breeding programs and reintroduction to the wild or natural habitat help preserving rare and endangered species. Every zoo accredited by AZA (Association of Zoos and Aquariums), partners with conservation organizations for collaborative research efforts towards species extinction.

Biodiversity conservation is a complex target that involves the participation of various stakeholders. Herein, zoos collaborate with government and non- government institutes and other researchers in various projects. The future of modern zoos will be determined by their ability to meet sustainable societal and environmental standards. Their goals should emphasize conservation by focusing on scientific research. However, some zoos have been criticized for prioritizing entertainment over animal welfare, while others argue that keeping animals captive is unethical. Despite these challenges, zoos continue to play an important role in the management, health, welfare and conservation of biodiversity.

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Original Article

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Fungal Pathogens Prevalence in Avian Species: Regional and Species-specific Variations

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ABSTRACT

Fungal pathogens are opportunistic, causing infections in caged and free-living birds with hypo-immunity. **Objectives:** To investigate the prevalence of fungal infections in avian species living in free and caged environments. **Methods:** The samples (n=600) were obtained using the simple random sampling technique (to ensure unbiased selection) from free-living and cage birds of Sindh Province and Azad Jammu and Kashmir, Pakistan. The samples were cultured on Sabouraud dextrose agar, Czapek dox agar and Potato dextrose agar and biochemical profiles. The cultures were subjected to biochemical profiles including cyclo-hexamine resistance, casein hydrolysis, fatty acid esterase activity and cellulose hydrolase activity. **Results:** Results showed that 4.16% of the samples were positive for the fungal species. Four fungal species including Aspergillus fumigatus (40.00%), Candida albicans (28.00%) Cryptococcus neoformans (24.00%) and Macrorhabdus ornithogaster (8.00%) were detected in the avian species. Significant ($p<0.05$) difference in fungal infection observed in free-living and cage birds. **Conclusions:** It was concluded that the fungal pathogens were detected in free-living and caged bird samples obtained from Sindh and Azad Jammu and Kashmir, Pakistan. The variation in infection rates among bird types and locations impacts living and environmental conditions on fungal susceptibility. It offers significant insights into fungal infections in birds and contributes to developing infection management and environmental well-being strategies.

INTRODUCTION

Fungal infections cause severe health problems to birds, whether they travel freely or caged. Fungal diseases may impact the entire population not just individual birds causing severe mortality and morbidity. To understand the epidemiology of infections in birds, it is necessary to examine the factors contributing to fungal infections in birds across different locations and occurrences. In birds, the fungal infection epidemiology is influenced by environmental factors, species susceptibility, immunological conditions and age. Illness severity and prevalence have a substantial impact on environmental conditions [1]. Fungal diseases have gained more epidemiologic attention as a consequence of the rise in fungal infections, which led to a rise in morbidity and mortality as well as significant diagnostic and treatment issues in healthcare [2]. Aspergillosis is one of the most common and harmful fungal infections in birds caused by Aspergillus species. Aspergillosis outbreaks in caged birds can be caused by inadequate ventilation, high humidity, and filthy conditions. The disease affects immunocompromised and stressed birds. In free-living birds, Aspergillus spores are commonly found in natural habitats, particularly in decaying organic matter and moist environments, which increases the risk of exposure [3]. Aspergillosis is primarily a respiratory disease, birds inhale the fungus spores and become infected, which can cause chronic or acute symptoms [4]. Candida infections are another common detrimental health issue especially,

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caused by Candida albicans in birds [5]. Free-living birds are more vulnerable to Candida infections transmitted by contaminated food or water sources; in areas where human activity has altered the natural ecosystem [6]. In caged birds, the most common transmission of this infection is usually linked to poor diet, stress and lack of husbandry practices [7]. The fungus Cryptococcus neoformans caused by Cryptococcosis is another respiratory ailment of domesticated and wild birds. This fungal infection is mostly spread by bird droppings and the transmission of illness in birds by inhaling spores of infected fouled soil [8]. Macrorhabdus ornithogaster causes macro-orhabdosis in birds which affects the gastrointestinal tract in budgerigars and finches (small passerines). Clinical symptoms of these infections include poor feather condition, diarrhea, and weight loss [9]. Risk factors like the host immune system, husbandry practices and climate have influenced fungal infection prevalence which varies by geographic location and avian species. Recently, the burden of fungal infections in domesticated and wildlife birds has increased due to bacterial resistance, emphasizing the need for preventive techniques, and improved therapeutic and diagnostic approaches in avian medicine. There is a gap in understanding the epidemiology of fungal infections in caged and free-living birds in Pakistan. Therefore, this study was conducted in two regions of Pakistan including Sindh and Azad Jammu & Kashmir to study the epidemiology of fungal infections in caged and free-living birds.

This study aims to explore the burden of fungal infections, fungal organisms and their susceptibility rate in both caged and free-living birds.

METHODS

A total of 600 (Faecal, cloacal, conjunctival, oropharyngeal/ tracheal swabs and blood) samples were obtained randomly from caged and free-living birds of regions; Hyderabad, Thatta, Badin, Dadu, and Karachi, Sindh Province, and Mirpur, Bhimber and Kotli, Azad Jammu and Kashmir. A simple random sampling method was used for data collection. This strategy provided each bird with an equal opportunity for selection (unbiased selection). The sample size was determined using the formula n=Z2P (1–P)/d2n, in which d is the margin of error (5%), P is the estimated prevalence of the target condition in the population (assumed 50% for maximum variability), and Z is the value for the desired confidence level (1.96 for 95%) [10]. Accordingly, a minimum of 384 samples were needed; however, 600 samples were gathered to ensure sufficient representation. At the time of collection, the physical condition, signs, and symptoms of the sampled birds were recorded. Fecal samples were collected aseptically from the cloacal region using cloacal swabs/sterile tubes, while for nasal, tracheal, and conjunctival samples, sterile cotton wool swabs containing phosphate buffer saline (PBS) were used to maintain the pH (Table 1).

Table 1: Sample Collection from Caged and Free-Living Avian Species

Type of Samples	Area	Caged/ Commercial Exotic Birds	Free-Living Birds	Total
Fecal, Cloacal, Conjunctival, Oropharyngeal/ Tracheal Swabs and Blood Samples	Hyderabad	50	25	75
	Thatta	50	25	75
	Badin	50	25	75
	Dadu	50	25	75
	Karachi	50	25	75
	Mirpur	50	25	75
	Bhimber	50	25	75
	Kotli	50	25	75
Total		400	200	600

Carefully, with the consent of owners, samples were taken from commercially sold healthy and sick pet avian species such as Budgies (Melopsittacus undulatus), Canaries (Serinus canaria domestica), Cockatiels (Nymphicus hollandicus), Crimson rosella (Platycercus elegans), Fisher (Agapornis fischeri), Lutino (Melopsittacus undulates), Pahari parakeet (Psittacula eupatria), Partridge (Rollulus rouloul), Pigeons (Columba livia domestica) and Quail (Synoicus ypsilophorus) for screening of fungal pathogens. During the collection of samples from pet birds, they were handled carefully to avoid causing harm or stress to the birds. The samples from free-living birds including backyard chicken (Aseel, Desi, Golden Missri and Sindhi), Bulbul (Pycnonotidae), Crow (Corvus), Dove (Columbidae), Duck (Bucephala albeola), Geese (Anser anser domesticus), Myna (Acridotheres tristi), Peacock (Pavo cristatus), Quail (Synoicus ypsilophorus) and Sparrow (Passer domesticus) were obtained using fog nets for about 09-12 hours/day in each area. The nets were inspected every hour for 06 consecutive days. The number of leaks losses and deaths in total were included. In addition, for catching free-living birds got services from experienced local bird predators. During the collection of samples from free-living birds, they were handled carefully to avoid causing harm or stress to the birds. The collected samples were brought in a cold chain container to the Department of Veterinary Microbiology, Sindh Agriculture University, Tandojam and transferred to the Veterinary Research Institute Peshawar, Khyber Pakhtunkhwa, for further processing. For surveillance of fungal infections, nasal, fecal, tracheal, and conjunctival swabs were screened by placing samples on Sabouraud dextrose agar (SDA) (Sigma-Aldrich), Czapek Dox agar (CDA) (Sigma-Aldrich) and Potato Dextrose Agar (PDA) (Sigma-Aldrich), using methods described by Pena *et al.*, [11]. Each anhydrous SDA 65gm/CDA 49gm/PDA 39gm were dissolved in 1000 ml of distilled water using magnetic

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stirrer MSH 300 (HVD, Life Science) prepared separately. The media was autoclaved at 121oC 15 lb/in^2 for 15 minutes. The media was cooled at room temperature and poured into Petri dishes. Following the sample (s) streaking/ cultured on Petri dishes and were incubated at 22oC for 24 hours. The cultural and colonial characteristics were observed for the occurrences of pathogens. Purification of culture was done by sub-culturing of typical well-separated colony on the corresponding medium. The process was repeated several times. The purity of the culture was checked by examining the stained smear. Smear was made from each type of colony, and identification of fungal species was performed based on morphological and lacto phenol cotton blue (LPCB) staining methods. The fungal growth and colony characteristics were observed and processed for further confirmation using biochemical analysis. The biochemical profiling including cycloheximide resistance, casein hydrolysis, fatty acid esterase activity and cellulose hydrolase was performed for the isolation and identification of fungal species in the free-living and caged bird samples. The PDA medium was prepared and inoculated with samples i.e. Control Plate: without cycloheximide and a test Plate: containing cycloheximide (concentration of 0.05% to 0.1%). Both plates were incubated at 25-30°C for 2-7 days. Growth was observed on the control plate but no growth on the test plate indicates cycloheximide-sensitivity. CDA media (concentration 15 mg/ml) inoculated with the samples and plates were incubated at 25–30°C for 14 days. The casein hydrolysis activity was determined by the formation of a clear zone around colonies. This proteolytic activity was an indicator (markedly clear zone) of the presence and differentiation of fungal pathogens. The agar plates were added 10 mM p-nitro phenyl acetate (p-NPA). The plates were inoculated with colonies and incubated at room temperature. The formation of a clear halo around the colony indicated high esterase activity, where the enzyme has broken down the p-NPA substrate. This activity could be noticed by the yellow-coloured p-nitro phenol product, as a halo around the fungal colony. Fungal samples were inoculated on the carboxy-methyl cellulose (CMC) agar plates. The plates were flooded with 0.1 (W/V) Congo red solutions and incubated at 30°C for 48-56 hours. Cellular activity was observed by the formation of clear zones around the fungal colonies. Statistical analysis was performed using Statistical Package for Social Science (SPSS) commercial software packages (version 17). The chi-square test was applied among the datasets to know the significant difference in prevalence (%.). p-value (probability value) is a measure which helps determine the significance of results in a hypothesis test. A value of $p<0.05$ was considered significant. A statistically significant difference in prevalence rates between caged and free-living birds was indicated by a p-value<0.05. This suggested that true changes in infection rates across different bird groups were reflected in the variance in prevalence rate, which were not the result of chance. A non-significant difference in the prevalence rates between caged and free-living birds was indicated by a p-value>0.05. This implies that regional differences in infection rates were statistically insignificant and most likely the result of chance.

RESULTS

In this study, 600 samples were examined for detection of fungal infections; out of these screened samples, 25 samples were detected positive for fungal pathogens. The rate of prevalence was calculated as 4.16% of infections in caged and free-living birds. The fungal pathogens were diagnosed positive in 17 (4.53%) and 08 (3.55%) samples in caged and free-living birds respectively (Table 2).

Table 2: Overall Prevalence of Fungal Infections in Caged and Free-Living Birds

Category	Samples Examined	Positive Samples	Frequency (%)
Caged Birds	400	17	4.53%
Free-Living Birds	200	08	3.55%
Total	600	25	4.16%

Data analyses indicated that 17 samples were recorded positive for fungal pathogens in caged birds. Among the analyzed samples obtained caged birds from Badin (n=2/50), Hyderabad (n=3/50), and Karachi (n=5/50) districts were detected positive for fungal organisms in Sindh province. The prevalence rate was calculated as 6.00%, 4.00% and 10.00% in districts Hyderabad, Badin and Karachi, respectively. The samples screened from Azad Jammu Kashmir; Bhimber (n=1) and Kotli (n=3) showed the presence of the pathogens. The prevalence rate was calculated as 2.00% and 6.00% in districts Bhimber and Kotli, respectively. A non-significant (p>0.8787) difference was observed in area-wise detection of fungal infections in caged birds (Table 3).

Table 3: Area-wise Detection of Fungal Infections in Caged Birds

Locations		Number of Samples Examined	No. of Positive Fungal Samples	Frequency (%)	X^2 (p-value)
Sindh	Hyderabad	50	3	6.00%	0.8787
	Thatta	50	2	4.00%	
	Badin	50	2	4.00%	
	Dadu	50	1	2.00%	
	Karachi	50	4	8.00%	
AJK	Mirpur	50	1	2.00%	
	Bhimber	50	2	4.00%	
	Kotli	50	2	4.00%	
Total		400	17	-	

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Data indicated that the samples (n=8) were diagnosed positive for fungal organisms in free-living birds. Among these, samples from Thatta (n=3), Badin (n=1) and Karachi (n=2) districts showed the presence of the pathogens. The samples from Bhimber (n=2) and Kotli (n=1) districts of Azad Jammu Kashmir had confirmed fungal species. The prevalence rate of the pathogens in birds was calculated as 8.00% and 4.00% in districts Bhimber and Kotli. A non-significant (p>0.5659) difference was observed in the area-wise detection of fungal infections in free-living birds (Table 4).

Table 4: Area-wise Detection of Fungal Infections in Free-living Birds

Locations		Number of Samples Examined	No. of Positive Fungal Samples	Frequency (%)	X^2 (p-value)
Sindh	Hyderabad	25	2	8.00%	0.5659
	Thatta	25	1	4.00%	
	Badin	25	0	0%	
	Dadu	25	0	0%	
	Karachi	25	2	8.00%	
AJK	Mirpur	25	0	0%	
	Bhimber	25	1	4.00%	
	Kotli	25	2	8.00%	
Total		200	8	-	

Four fungal species were identified from caged and free-living birds sampled. Aspergillus fumigatus (10), Candida albicans (07), Cryptococcus neoformans (06) and Macrorhabdus ornithogaster (02) were identified in the samples obtained from avian species. The highest prevalence rate (40.00%) was recorded for Aspergillus fumigatus followed by Candida albicans (28.00%), Cryptococcus neoformans (24.00%) and Macrorhabdus ornithogaster (8.00%). Statistically, the difference in the prevalence rate of fungal organisms detected in caged and free-living birds was significant (p<0.0017) (Table 5).

Table 5: Fungal Species Detected in the Samples of Caged and Free-living Birds

Infected Avian Species	Fungal Organisms	Pathogen Occurrence	Frequency (%)	X^2 (p-value)
Quail, Canaries, Cockatiels, Budgies, Pahari parakeets, Pigeons, Myna, Duck, Geese, Backyard chicken	Aspergillus fumigatus	10	40.00%	0.0017
Quail, Cockatiels, Lutino, Backyard chicken, Pahari parakeet	Candida albicans	07	28.00%	
Canaries, Pahari Parakeets, Pigeons, Dove	Cryptococcus neoformans	06	24.00%	
Cockatiels, Budgies	Macrorhabdus ornithogaster	02	8.00%	
Total		25	- -	

Data analyses indicated that the susceptibility rate of fungal infections was higher in cockatiels and Pahari parakeets (23.52%) compared to pigeons (17.64%), canaries (11.76%), budgies (11.76%), quail (5.88%) and lutino (5.88%). Statistically, the difference in susceptibility rate of fungal infections in different caged birds in different regions of local commercial markets was non-significant (p>0.2174) (Table 6).

Table 6: Susceptibility rate of Fungal Infections in Different Caged Birds in various Regions of Local Commercial Markets

Caged Birds	No. of Positive Fungal Samples	Susceptibility Frequency (%)	X^2 (p-value)
Quail (Synoicus Ypsilophorus)	Aspergillus Fumigatus (1)	5.88%	0.2174
Canaries (Serinus Canaria Domestica)	Cryptococcus Neoformans (1) Aspergillus Fumigatus (1)	11.76%	
Cockatiels (Nymphicus Hollandicus)	Aspergillus Fumigatus (1) Candida Albicans (1) Macrorhabdus Ornithogaster (1) Candida Albicans (1)	23.52%	
Budgies (Melopsittacus Undulatus)	Aspergillus Fumigatus (1) Macrorhabdus Arnithogaster (1)	11.76%	
Pahari Parakeet (Psittacula Eupatria)	Aspergillus Fumigatus (1) Cryptococcus Neoformans (2) Candida Albicans (1)	23.52%	
Partridge *(Rollulus Rouloul)*	0	0%	
Fisher (Agapornis Fischeri)	0	0%	
Pigeons (Columba Livia Domestica)	Aspergillus Fumigatus (1) Cryptococcus Neoformans (2)	17.64%	
Lutino (Melopsittacus Undulates)	Candida Albicans (1)	5.88%	
Crimson Rosella (Platycercus Elegans)	0	0%	-
Total	17	-	-

Statistically, the difference in susceptibility rate of fungal infections in different caged birds in different regions of local commercial markets was non-significant (p>0.05). The susceptibility rate of fungal infections was higher in Backyard chicken (37.50%) compared to duck (25.00%), dove (12.50%), myna (12.50%) and geese (12.50%). Statistically, the difference in susceptibility rate of fungal infections in different free-living birds in different regions of local commercial markets was non-significant (p>0.1791) (Table 7).

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Table 7: Susceptibility rate of Fungal Infections in Free Living Birds in various Regions

Free-Living Birds	No. of Positive Fungal Samples	Susceptibility Frequency (%)	X^2 (p-value)
Quail (Synoicus Ypsilophorus)	0	0%	0.1791
Dove (Columbidae)	Cryptococcus Neoformans (1)	12.50%	
Bulbul (Pycnonotidae)	0	0%	
Crow (Corvus)	0	0%	
Sparrow (Passer Domesticus)	0	0%	
Myna (Acridotheres Tristi)	Aspergillus Fumigatus (1)	12.50%	
Peacock (Pavo Cristatus)	0	0%	
Duck (Bucephala Albeola)	Aspergillus Fumigatus (1) Candida Albicans (1)	25.00%	
Geese (Anser Anser Domesticus)	Aspergillus Fumigatus (1)	12.50%	
Backyard Chicken	Aspergillus Fumigatus (1) Candida Albicans (2)	37.50%	
Total	8	-	-

In this study, the most common risk factors associated with fungal infections in different caged and free-living birds were determined based on external environmental conditions. In the case of caged birds; housing, husbandry, humidity control, feed and water, quality and human interaction at commercial places were determined risks associated with the rate of fungal pathogens. Free-living birds may have differences in infection rate due to variations in external environmental factors including humidity and elevated temperature in Sindh Province, and heavy snowfall in Jammu and Azad Kashmir, as well as, individual conditions such as exhaustion, weakened immunity and exposure to contaminated feed residues.

DISCUSSION

The prevalence of fungal infections in the present study was estimated as 4.16% in caged and free-living avian species. Our findings are from a previous study A. fumigatus was detected in 30% of house sparrows [12] and 6-13% in captured pink-footed geese, Canada geese or herring gulls that presented as healthy carriers [13]. It has been demonstrated that the proportion of free-living birds in wild passerines was around 2.9% [14]. Discrepancies in prevalence rate are associated with study design, diagnostic methodology, sample size, environmental conditions, management approaches, and cage hygiene [15]. Several factors such as poor ventilation, high density and limited space in caged birds may pose higher infection rates. On the other hand, free-living birds may face a variety of challenges including exposure to naturally occurring fungal spores and environmental conditions that might alter infection rates [16]. In current research, four fungal species were identified from caged and free-living birds. Among these, the most prevalent was Aspergillus fumigatus followed by Candida albicans, Cryptococcus neoformans and Macrorhabdus ornithogaster. Findings are consistent with studies, which stated that Aspergillus fumigatus was a more prevalent pathogen in farmed and wild birds. Avian respiratory illnesses are mostly caused by Aspergillus fumigatus as reported by [17, 18]. According previous study demonstrated that occurrences of Candida albicans in mucosal surfaces and avian guts may increase in response to immunosuppression or stress conditions of birds [19]. Similarly, it has been observed that C. neoformans incidence was higher in several bird species [20]. The discrepancy might be explained by differences in specific bird populations studied, environmental variables, and geographical locations. Ornithogaster is a substantial pathogen, and its frequency is typically lower than that of other widely dispersed fungi such as A. fumigates [21]. In caged birds, the susceptibility to fungal infections was recorded higher in Cockatiels and Pahari parakeets than pigeons, canaries, budgies, quail and lutino. Similarly, in free-living birds, susceptibility to fungal infections was exhibited higher in Backyard chickens than ducks, doves, mynas and geese. The findings of this study are consistent with previous research that observed that pigeons are more prone to become infected due to their high density and continual interaction with secondary infections [22]. Cockatiels and Pahari parakeets are sensitive to stress and environmental conditions, which make them more prone to fungal infections [23, 24]. According to a former study, fungal infection susceptibility in many birds depends upon factors such as genetic predisposition, environment and diet [25]. Some bird species are more susceptible to infections due to factors such as close quarters, immune system differences, and environmental stress. Our results are supported by previous studies, which reported lower rates of fungal infections in canaries and other similar species [26]. Previous research studies demonstrated that quail and duck habitat conditions render them more susceptible to fungal infections [27, 28]. A prevalence of 1.7 to 3.1% was detected in grey herons, mallards and coot birds living in Guadalquivir marshes [29]. Aspergillus was detected in 9–50% and 27–31% of nocturnal heron chicks [30]. This fungal disease was recognized as the primary cause of death for 6 to 23% of common loons [31]. Aspergillosis was diagnosed in 31% of necropsied birds, mostly herring gulls [32].

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CONCLUSIONS

It was concluded that the fungal pathogens were detected in free-living and caged bird samples obtained from Sindh Province and Azad Jammu and Kashmir, Pakistan. Aspergillus fumigatus, Candida albicans, Cryptococcus neoformans and Macrorhabdus ornithogaster were detected in avian species. The variation in infection rates among bird types and locations impacts living and environmental conditions on fungal susceptibility. The research is valuable for the prevalence and distribution of fungal pathogens in avian species, emphasizing the mode of infection rates and environmental factors variation. It offers significant insights into fungal infections in birds and contributes to developing infection management and environmental well-being strategies.

Authors Contribution

Conceptualization: SHA, RA
Methodology: AMUD, MS
Formal analysis: AMUD, SHA, DHK, RA
Writing review and editing: AMUD, SHA, DHK, MS, RA

All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

All the authors declare no conflict of interest.

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Comparative Study of Herbal Feed Additives on Growth Performance and Haematology in Female Dairy Calves

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ABSTRACT

Herbal feed additives, including turmeric (*Curcuma longa*), garlic (*Allium sativum*), and ginger (*Zingiber officinale*), are increasingly explored for their potential to enhance livestock performance. **Objective:** To evaluate the effects of *Curcuma longa* (Turmeric), *Allium sativum* (Garlic) and *Zingiber officinale* (Ginger) powder on the growth performance and hematological values of cross-bred female cow calves. **Methods:** : This experimental study was conducted from June, 2024 to October, 2024.The experimental groups were fed with standard rations and different concentrations of feed additives, i.e. 0.5 %, 1% and 1.5% of calf starter for sixteen weeks. An automated haematology analyzer was used to carry out haematological studies. **Results:** This study revealed a significant increase in weight gain along with PLT counts by the calves fed with *Allium sativum* (Garlic) powder at varying concentrations of 1.5 %, 0.5 % and 1.0 % calf starter, respectively. Haematological analysis showed substantial improvement in RBCs/WBCs/PLT counts and Hb/HCT levels in calves receiving 1.5% Curcuma longa (Turmeric) powder. No significant increase in growth rate was observed in any group fed with *Zingiber officinale* (Ginger) as a feed additive. **Conclusions:** On the basis of findings of this study, the use of 1.5 % *Allium sativum* (Garlic) as feed additive along with standard diet for increased weight gain in dairy calves is recommended. The addition of 1.5 % Curcuma longa (Turmeric) as feed additive in the diets of dairy calves also leads to increased count of RBCs, Hb, HCT, PLT and WBC's values resulting into better immunity and resistance.

INTRODUCTION

Nutrition is critical to the growth and overall health of animals, particularly in their early stages of life. Cow calves require adequate nourishment for healthy growth and the development of strong immunological and haematological systems. Traditionally, synthetic feed additives have been employed to increase cattle production; however, there is increased interest in the use of herbal feed additives due to their natural origins and possible health benefits. Research on early life nutrition is increasingly recognized as vital, given that optimizing the development of healthy dairy calves is essential for ensuring long-term financial sustainability in dairy farming. The period of growth between birth and weaning is extremely important for economic farming [1, 2]. Early life growth rate has long-term impacts on age at first calving, future milk output, and lifespan [3]. Calves are vital to future dairy herds, and maintaining optimal growth and early puberty is critical to their development. While milk-feeding boosts weight gain, lowers sickness, and encourages natural behavior, it is not economically viable as a primary diet. Growth, rumen development, feed efficiency, and overall dairy performance are all highly impacted by weaning techniques [4]. Feed is an essential component for calves as it accounts for 60–70% of production expenses. Hormones and antibiotics were formerly used to boost growth, but the demand for substitute feed additives has grown due to concerns about their use. Medicinal plants and their derivatives, known as phytobiotics, can positively

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improve animal rumen fermentation, performance, and overall health, depending on the individual components revealed that phytogenic are a group of natural growth promoters or non-antibiotic growth promoters used as feed additives, derived from herbs, spices or other plants [5, 6]. Positive results during the suckling period have been found in recent research on the use of herbal supplements in ruminants [7-9] noted that using herbal formulas as a supplement could lead to feeds that don't contain antibiotics. During the nursing period, herbal extracts can maintain or improve calf health and productive parameters [10, 11]. In dairy and cattle production systems, herbal formulations are being researched as a means of increasing feed efficiency and as an alternative to antibiotics during illness [12-14]. Garlic (*Allium sativum*) is being used as a spice and traditional medicine with a high nutritional value. It is a rich source of calcium and phosphorus and also has high carbohydrate content [15]. The active components comprise of allicin (dialthiosulfinate), and n-acetyl cysteine. Garlic promotes vasodilation and nitric oxide activity, which helps to reduce blood pressure which has also been reported in several studies. Current research has revealed that garlic has antibacterial, antifungal, antioxidant, anticancer and antidiabetic effects. Turmeric (*Curcuma longa*) is frequently utilized in Asian cuisines and traditional medicine as it is an herbaceous spice. It is also well-known for its vivid color, added to food as a stabilizing and coloring agent [16]. Turmeric consists of advantageous phenolic compounds such as Curcumin, bisdemethoxycurcumin, and demethoxycurcumin due to which it's referred as strong antioxidant, nematocidal, anti-inflammatory, anti-carcinogenic, and anti-hepatotoxic qualities [17-22]. Ginger (*Zingiber officinale*) is a extensively used herb with anthelmintic properties which have been studied in invitro and in vivo experiments [23-26]. It's a popular culinary spice and medicinal plant [27]. Ginger is also known for its antioxidant, antibacterial, antiviral, anthelmintic, and anti-diabetic properties [28]. During drying process of ginger, a potent compound called Shogaol is formed which contributes to its medicinal effects, including both antineoplastic and anti-inflammatory benefits [29, 30] Studies have indicated that these herbs may positively influence growth performance and health in livestock, yet there is limited research specifically focusing on their effects on cow calves. This study aims to evaluate the effects of turmeric, garlic, and ginger powders as economical and natural feed additives on the growth performance and hematological profiles of crossbred female cow calves over an 8-week period. Understanding the effects of herbal feed additives could offer a natural alternative to synthetic supplements, potentially leading to improved health and productivity in dairy cattle. This research holds significance not only for enhancing livestock management practices but also for advancing sustainable and health-conscious approaches in animal husbandry.

METHODS

Procurement of the Feed Additives

Fresh garlic (*Allium sativum*), turmeric (*Curcuma longa*) and ginger (*Zingiber officinale*) powder were obtained from local market and then dried. After drying the husks were separated and the bulbs were ground to powder by electric grinder.

Study Design

This experimental study was conducted from June, 2024 to October, 2024, to evaluate the effects of turmeric (*Curcuma longa*), garlic (*Allium sativum*) and ginger (*Zingiber officinale*) as feed additives on the growth performance and hematological parameters of female cow calves. A total of 50 cross bred female cow calves, 8 weeks old having uniform body weight (24 Kg) from a local dairy farm were selected for this study. These calves were divided in ten groups comprising of one control and three experimental groups. Group A was considered as control and B, C and D were experimental containing fifteen calves in each group. Group B, C and D will be further divided into three sub groups as B1, B2, B3, C1, C2, C3, D1, D2 and D3. Each sub group will comprise 5 experimental calves. Control group was given standard feed without any feed supplement. The experimental groups were as follows:

Group B1, B2 and B3 were given standard feed with addition of Curcuma longa at the rate of 0.5%, 1%, 1.5 % of total quantity of calf starter respectively.

Group C1, C2 and C3 were given standard feed with addition of *Allium sativum* at the rate of 0.5%, 1%, 1.5 % of total quantity of calf starter respectively.

Group D1, D2 and D3 were given standard feed with addition of *Zingiber officinale* at the rate of 0.5%, 1%, 1.5 % of total quantity of calf starter respectively.

Feeding Schedule and Calculations

Each calf was provided with accurately weighed feed twice daily, once in the morning and once in the evening. Clean drinking water was offered ad- libitum throughout eight weeks.

The standard feed provided to each calf, at different stages of age has been detailed in table 1.

Table 1: Sample Collection from Caged and Free-Living Avian Species

Age (Weeks)	Milk Replacer (Lit)	Calf Starter (Kg)	Concentrate (Kg)	Green Fodder (Kg)
9	5	1.30	-	1.05
10	4	1.60	-	1.05
11	4	1.80	-	1.05
12	3	2.10	-	1.20

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13	3	2.40	-	1.20
14	2	2.50	0.1	1.20
15	2	2.60	0.2	1.20
16	2	2.70	0.3	1.20

Growth Performance Evaluation

The calves were individually weighed at weekly intervals to monitor their growth performance. Data were collected and calculated as follows: Body Weight Gain (BWG) = Final weight -Initial weight.

Hematological Investigation

Blood samples were collected from control and experimental groups on 16th weeks age. A total of 1.5 ml blood was drawn from jugular vein of all the calves using sterile plastic syringe. It was transferred to sterile EDTA coated vials and transported under refrigeration to research lab of Lahore Garrison University. Samples were labelled properly and samples were processed in three replicates for control and experimental groups. Automated hematology analyzer (BIOBASE Auto Hematology Analyzer Model BK- 5000 Jinan, China) was used to carry out hematological studies.

Statistical Analysis

Data was analyzed using one-way ANOVA with IBM SPSS. LSD test was employed to compare variances within means at the significance level of $P<0.05$.

RESULTS

Growth Performance

At the end of the 16th week of the trial, it was observed that the live body weight (kg) of crossbred female cow calves receiving 1.5% Allium sativum powder supplementation, along with standard feed, showed a highly significant increase as compared to the control group. This suggests that the Allium sativum supplementation had a strong positive effect on growth performance. Additionally, calves fed with 1.5% Curcuma longa powder also exhibited a significantly positive effect on their growth rate. This indicates that Curcuma longa supplementation was effective in enhancing growth performance as well. In contrast, calves receiving 0.5% Allium sativum and varying concentrations (0.5%, 1%, and 1.5%) of Zingiber officinale showed a significantly negative effect on their growth rate. This suggested that these treatments were not effective in promoting growth and might have had a detrimental impact. The weekly body weight gains of the control and experimental groups are further illustrated in figure 1.

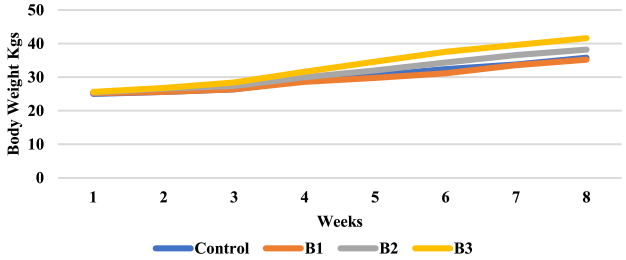

Figure 1: Comparison of Weekly Body Weight Gain of Control and Experimental B Group of Calves

In figure 2 the experimental groups supplemented with *Allium sativum* (C1, C2, C3) exhibited improved growth performance compared to the control group. Among these, C3 (1.5% *Allium sativum*) showed the most significant weight gain, reaching 47.8 kg by the end of the study period. These results highlighted the effectiveness of Allium sativum as an herbal feed additive for enhancing growth in crossbred female cow calves.

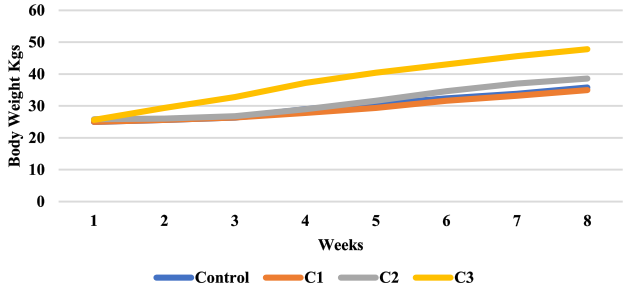

Figure 2: Comparison of Weekly Body Weight Gain of Control and Experimental C Group of Calves

In figure 3, the experimental groups supplemented with *Zingiber officinale* (D1, D2, D3) displayed less pronounced growth. Minimal improvements were observed compared to the control group, indicating the limited effectiveness of *Zingiber officinale* in promoting weight gain in crossbred female cow calves.

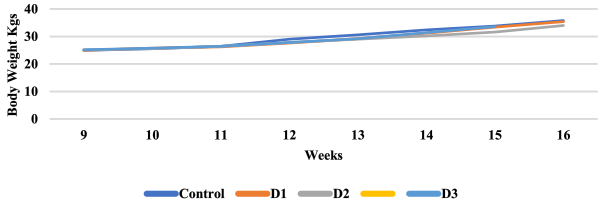

Figure 3: Comparison of Weekly Body Weight Gain of Control and Experimental D Group of Calves

Table 2 revealed that 1.5% *Allium sativum* (C3) and 1.5% Curcuma longa (B3) significantly increased body weight compared to the control, with p-values of 0.000 and 0.0002, respectively. In contrast, other groups, including those with *Zingiber officinale* and lower doses of *Allium sativum* and *Curcuma longa*, showed no significant differences or negative effects. At the end of 16th week age, the total body weight gains of all groups indicated maximum gain by C3 followed by B3 and C2 groups.

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Table 2: Statistical Comparison of All Experimental Groups Body Weight with Control Group

Groups	Groups	Difference	Standard Deviation	Mean ± SEM	p-Value
Control	B1	0.600	1.3038	35.2 ± 0.58	1.000
	B2	-2.400	1.3038	38.2 ± 0.58	0.513
	B3	-5.800*	1.8166	41.6 ± 0.81	0.0002
	C1	0.800	1.0000	35.0 ± 0.44	0.999
	C2	-2.800	2.7928	38.6 ± 1.24	0.302
	C3	-12.000*	1.3038	47.8 ± 0.58	0.000
	D1	0.400	1.3416	35.4 ± 0.60	1.000
	D2	1.800	1.5811	34.0 ± 0.70	0.839
	D3	0.200	2.0736	35.6 ± 0.92	1.000

Hematology

The hematological values of red blood cells (RBCs), Hemoglobin (Hb), hematocrit (HCT), platelets (PLT) and white blood cells (WBCs) of all the calves of control and experimental groups obtained at sixteen-week age using automated hematology analyzer (BIOBAE).

RBCs

In figure 4 at the end of the 16th week, the RBC values showed the greatest increase in the B3 group, followed by D3 and C2. The 1.5% Curcuma longa feed additive demonstrated significant positive effects on the RBC count in crossbred female cow calves. Additionally, a notable increase in RBC values was observed in calves fed with 1.5% *Zingiber officinale* and 1% *Allium sativum*.

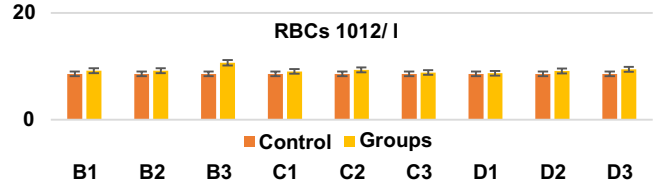

Figure 4: Comparison of RBC Values in Control and Experimental Groups of Calves

Hemoglobin

Significant (P<0.05) positive effects have been observed on the number of Hb values of crossbred female cow calves fed with 1.5% *Curcuma longa* as feed additive. The use of 1.5% *Curcuma longa* as feed additive resulted into improved number Hb values as shown in the figure 5. At the end of the 16th week, the Hb values showed the greatest increase in the B3 group, followed by C2 and C1.

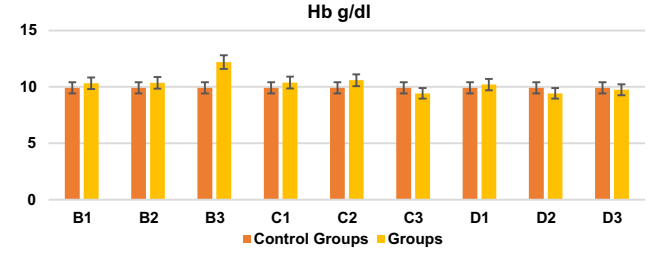

Figure 5: Comparison of Hb Values in Control and Experimental Groups of Calves

HCT

As shown in the figure 6, B3 group of experimental calves fed with 1.5% Curcuma longa powder is highly significant (P<0.05).

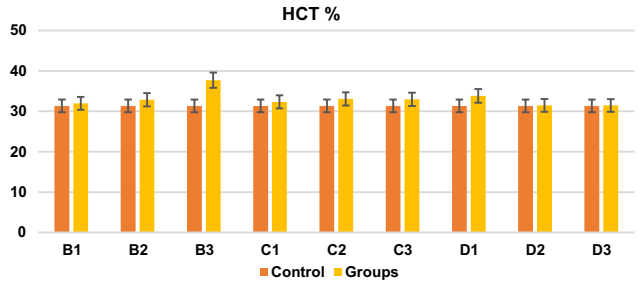

Figure 6: Comparison of HCT Values in Control and Experimental Groups of Calves

PLT

It was observed that B3 group of experimental calves fed with 1.5% *Curcuma longa* powder is highly significant (P<0.05) and resulted into increased number of PLT values. A significant positive effect in the calves fed with 1 % and 0.5 % Curcuma longa powder was also noticed. At the end of the 16th week, the PLT values demonstrated the greatest increase in the B3 group, followed by C2 and C1 (Figure 7).

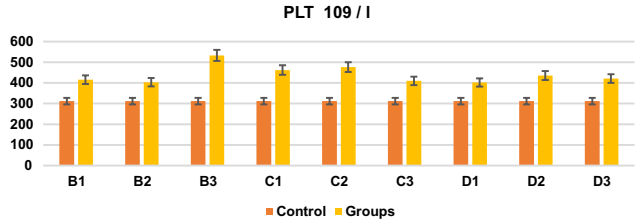

Figure 7: Comparison of PLT Values in Control and Experimental Groups of Calves

WBCs

Figure 8 showed that WBCs of experimental group of calves B3 fed with 1.5 % Curcuma longa powder have highly significant (P<0.05) effect and have assumed unequal variances. WBC's of B2, C3 and D2 experimental group of calves have insignificant effect and have assumed equal variances. At the end of the 16th week, the WBC values showed the greatest increase in the B3 group, followed by C1 and D1.

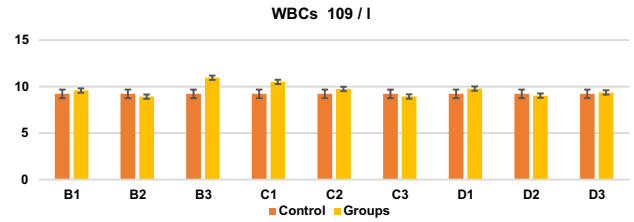

Figure 8: Comparison of WBC Values in Control and Experimental Groups of Calves

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DISCUSSION

The use of herbal feed additives in dairy calf nutrition gained attention in recent years as a potential strategy to improve growth performance and overall health. While research on herbal feed additives for dairy calves was ongoing to ensure calf growth, it remained important to provide them with a balanced diet that met their nutritional requirements. High-quality calf starter feed, proper colostrum intake, access to clean water, and appropriate management practices ensured comfort and were key factors in promoting healthy growth and development in calves. The present study targeted novel herbal feed additives to be used in calf feed while keeping the rest of the factors standardized. The effects of different concentrations (0.5%, 1%, and 1.5% of feed) of Curcuma longa, Allium sativum, and Zingiber officinale were studied for their impact on the growth performance and hematological values of crossbred female cow calves. The results demonstrated a highly significant ($P<0.05$) increase in weight gain in C3 (Allium sativum at the rate of 1.5%), followed by B3 (Curcuma longa at the rate of 1.5%) experimental groups of crossbred female cow calves. Similarly, RBC and WBC counts, Hb, and HCT content of experimental group B3 (fed with 1.5% Curcuma longa powder) showed a highly significant positive ($P<0.05$) effect. In contrast, the PLT count of experimental groups B3 (fed with 1.5% Curcuma longa powder), C1 (Allium sativum at the rate of 0.5%), and C2 (Allium sativum at the rate of 1.0%) exhibited a highly significant positive ($P<0.05$) effect. The results of the present study provided compelling evidence for the positive effects on the growth rate and hematological values of crossbred female cow calves. These findings demonstrated a direct correlation between the growth rate of calves and the use of 1.5% Allium sativum as a feed additive. The use of 1.5% Curcuma longa as a feed additive also resulted in an improved number of RBCs, Hb, HCT, PLT, and WBC values. In agreement with these findings, it was found that the improvement in body weight gain was caused by the addition of garlic powder in feeding practices [13]. According to previous research, garlic had a high nutritional value, was a rich source of calcium and phosphorus, and contained high levels of carbohydrates [31]. This aligned with the present study, where the positive effect of 1.5% Allium sativum as a growth promoter was observed. Supporting evidence suggested that garlic had the ability to improve growth rate, digestibility, and carcass traits in livestock production as an alternative growth promoter. It was reported that alliinase enzymes were the main components of garlic [17, 32]. Additionally, garlic was found to control infectious diseases and was used to prevent wound infections. In agreement with this study, the increased PLT count observed in the study indicated its role in early wound healing through blood clotting. Evidence supported the fact that regular consumption of garlic could reduce factors associated with cardiovascular diseases. Similarly, turmeric powder was reported to increase nutrient utilization in female crossbred calves during the winter season, whereas garlic powder supplied at 15g/day per calf in the diet improved growth performance and reduced feed costs [33]. This study also revealed that the use of 1.5% turmeric powder and 1.5% garlic powder had a positive effect on hematology and overall body weight gains up to sixteen weeks of age. These findings were in total agreement with investigations by Balamurugan et al. (2014), who revealed that crossbred calves receiving garlic supplements gained considerably more overall body weight and average daily gain. It was reported that natural growth promoters such as garlic and ginger might be potential alternatives to commonly used chemical growth promoters like antibiotics [23]. However, in contrast to this study, the results revealed that the use of 0.5% garlic powder and 0.5%, 1%, and 1.5% ginger powder had no positive effect on the overall body weight of calves. It was reported that supplementation of garlic in crossbred calves increased average daily gain by 5.20% [34]. The increased average daily weight gain values observed in these findings suggested the positive role of garlic in growth performance. It was demonstrated that turmeric oil supplementation had a significant impact on all hematological parameters, including packed cell volume, hemoglobin, red blood cell count, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, white blood cells, and their differentials [35]. In this study, supplementation with 1.5% turmeric powder resulted in an improved number of RBCs, Hb, HCT, PLT, and WBCs, thus having a significant positive effect on the hematology of crossbred female cow calves. According to Oyebanji et al. (2018), a diet supplemented with 10g/kg of turmeric was associated with considerably lower levels of total cholesterol, greater levels of high-density lipoprotein, and lower levels of low-density lipoprotein when compared to the control group. The study revealed improved hematology. Thus, improved hematological values coupled with lower blood cholesterol levels were expected to contribute to the good health of future herds. This study noted that the use of 1.5% turmeric powder resulted in an increased number of RBCs, Hb, HCT, and WBCs. These findings were endorsed by previous studies, which demonstrated that dietary

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supplementation with turmeric oil lowered cholesterol levels. It was reported that exposure to fenvalerate significantly decreased RBC counts, TLC counts, differential counts, PCV percentage, hemoglobin percentage, PCV, neutrophils, eosinophils, and monocyte levels. However, after turmeric treatment, there was a significant increase in these levels [36, 37], which aligned with the findings of the present study. In accordance with previous research, the concentration of platelets, hematocrit, monocytes, lymphocytes, and granulocytes increased significantly at 0.25% supplementation of Curcuma longa [38]. This study noted that the use of Curcuma longa powder at 1.5% as a feed additive significantly increased the total count of RBCs, hematocrit, platelets, and WBCs. This study was also in agreement with [23], who reported that Curcuma longa could enhance biochemical and hematological parameters in domestic animals when used as a feed additive. It was proposed that taking Curcuma longa at 50, 100, or 200 mg/kg body weight for the duration of the trial might improve hematological markers and overall health [39]. The study demonstrated the significant effect of 1.5% Curcuma longa powder in regulating blood parameters. These results contrasted with previous studies, which found that herbs like ginger had biological benefits, including promoting growth and stimulating the immune system [40-41]. In this study, the use of 0.5%, 1%, and 1.5% ginger powder did not show any significant effect on weight gain and hematological parameters compared with the control group. The reason might have been the use of different concentrations by the researchers. Although there was some evidence suggesting potential benefits, further studies were needed to determine the most effective herbal feed additives, optimal dosage levels, and their long-term effects on calf growth, health, and milk production.

CONCLUSIONS

Supplementation with herbal feed additives is proven to be a potential strategy to improve growth performance and blood parameters of calves. These additives offer several potential benefits to farmers for increased profitability. The use of economical herbal feed additives in appropriate concentration may lead to improved growth performance and overall health. On the basis of findings of these study the feeding of Allium sativum (Garlic) powder at the rate of 1.5 % of the total quantity of calf starter fed to female cross bred cow calves between 8 to 16 weeks of age has a positive effect on the body weight gain. The feeding of Allium Sativum (Garlic) powder at the rate of 0.5 % and 1 % of the total quantity of calf starter fed to female cross bred cow calves between 8 to 16 weeks of age has a positive effect on the PLT counts The feeding of 1.5% Curcuma longa (Turmeric) powder at the rate of 1.5 % of the total quantity of calf starter fed to female cross bred cow calves between 8 to 16 weeks of age has a positive effect on the RBCs, Hb, HCT, PLT and WBCs counts.

Authors Contribution

Conceptualization: FJ, MJK
Methodology: TZ
Formal analysis: HR
Writing, review and editing: TZ

All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

All the authors declare no conflict of interest.

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Investigation of Zoonotic Cestode (Hymenolepididae: Cyclophyllidea) from Rodents in Suburban Hyderabad: Prevalence and Public Health Risk

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ABSTRACT

The Rats and mice are well-known vectors of ecto and endo parasites and have zoonotic and veterinary importance. **Objectives:** To study two Hymenolepididae species in sub-urban rodents: Rattus rattus and Mus musculus and to analyse the elements contributing to their occurrence in the environment and causing sanitary risks and to evaluate the prevalence, mean, and abundance. **Methods:** 40 samples were collected including 21 rats and 19 mice captured from sub-urban areas of Hyderabad. Prevalence means and abundance was recorded with the help of Quantitative Parasitology-version 3.0. Morphological characters were studied using line diagrams and photographs of this cestode. Identification of cestode was done with key books and recent research papers. **Results:** Morphological analysis of hosts i-e Rattus rattus and Mus. musculus revealed that two cestode species (Hymenolepis diminuta, H. nana) were found in the sub-urban localities of the Hyderabad district. These localities are mostly under development, lack freshwater facilities, and have sanitary risks. This cestode is found in the small intestine. Morphometric studies were conducted on both species: H. nana and H. diminuta. Statistical value: Prevalence, mean, abundance, and mean intensity were calculated, also observed host species correlation with sex, localities, and season. **Conclusions:** It was concluded that cestode parasites have public health importance. Studies provide valuable data to local and provincial organizations and also help in the diagnosis of zoonotic diseases. This study also provides references to minimize the rodent population, especially in suburban areas where the sewage system is poor and zoonotic diseases are common.

INTRODUCTION

Helminthiases was neglected in about 20% of people affected in Latin- America, and throughout the world, more than 3800 people were infected [1]. These helminths infections are more prevalent in rural and overpopulated areas that are poorly constructed, and sanitation issues that cause environmental contamination. [2]. Previously, this helminthiases disease was cosmopolitan, especially in an anthropogenic environment. This disease was infested worldwide and was affected by cestode genera H. diminuta (Rudolphi, 1819) and H. nana (Von Siebold, 1852). These cestode species life cycles involve definitive hosts i-e humans, rats, and mice, and intermediate hosts and arthropods. The frequent host species around humans are rats, mice, and arthropods. The disease is more prevalent in young than adults and is mostly in marshy areas [3-5]. The rat and mice are definitive for H. diminuta (Rudolphi, 1819) species. For parasitosis, humans by complete life cycle transmitted from the intermediate host an arthropod [6]. Whereas, H. nana (Von Siebold, 1852) type host is humans, although it infested other mammals. Previously, around the world, this species infected more than 20 million by direct contact [7, 8]. Furthermore, epidemiologically seen higher infection in children of trashed and overpopulated areas [6]. The climate of Hyderabad is dry with little rainfall. In day time observed hot up to 40°C meanwhile, nights are pretty cold and airy [9]. In Pakistan, Hyderabad is the fifth largest city and in Sindh, it stands as the 2nd city of Province. It is estimated in the 2017 census of Pakistan that more than 13 million homes were included in Municipal Corporations and cantonments. Slum houses were not documented. After the 2022 flood, most affected were shifted to Hyderabad [10]. Therefore, the

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Hyderabad district seems favorable for urban rodents, the most frequent species were black rats (Rattus rattus Linnaeus, 1758) and house mice (Mus musculus Linnaeus, 1758). These hosts were bearing parasitic infections. In cestode mostly H. diminuta (Rudolphi, 1819) and H. nana (Von Siebold, 1852) parasitosis in definite hosts i-e hosts [11-14]. These two species are cosmopolitan, and distributed in trashed areas. No studies have been conducted on the family of Murids in Pakistan. Only a few authors have been given intentions at different times. [15-17]. A study on zoonotic and non-zoonotic parasites in Rattus rattus in the Sawat district of Pakistan has been conducted. Eight species were noticed, including H. diminuta and two other species of two species from Hymenolepis genus [18]. In another part of the country of Pakistan, Malakand has been studied for intestinal parasites in school children. About eight species were found including H. nana in stool samples of children [19]. However, the purpose of this study is to analyse zoonotic infection of Hymenolepididae species present in overpopulated and poor sanity areas of district Hyderabad. This study aims to evaluate the major reason for the Hymenolepididae species in these sub-urban hosts i-e House mice and black rats.

METHODS

Hyderabad district is located on the east bank of the Indus River, at 25.367 °N latitude and 68.367 °E longitude. It consists of four localities of approximately consist on 5 lac households and 2 and half lac populated districts. This study was conducted on four localities: Qasimabad, Latifabad, Hyderabad Sadar, and Hyderabad City. All studies conducted on sub-urban sides mainly focus on a higher level of trash areas, where houses have low hygiene and are overcrowded. Moreover, target slum areas that are poorly developed. These areas lack fresh water facilities and are forced to use higher containment water. The sample was collected from August 2021 to 2023 December. The Captured collected were divided into two periods: Summer-spring with an average temperature is 40°C high and low of 23°C and winter-autumn with an average temperature higher than 29°C and a low of 15°C. Rat and Mice were collected in a cage and brought to the parasitology lab of the Zoology Department of the University of Sindh Jamshoro. Hosts were dissected, visceral organs detached and placed separately in Patri Plates. The organs were perturbed in the body cavity and teased in normal saline. The visual and AmScope New Dual Lit 6W LED Trinocular Stereo Zoom microscope observation for the presence of Cestode in the small intestine of hosts. The cestodes were collected and stored in 70% alcohol. Specimens were stained with borax carmine, dehydrated with an alcohol series, and made permanent slides by using Canada balsam. Photomicrographs were taken with an OMAX 40X-2500X Trinocular Compound Microscope with a 10MP camera. Line drawings were drawn using a microscope Olympus ch 20 drawing tube. Measurements were in millimetres [20]. Identification by key books and research papers. Statistical value: Prevalence, mean, abundance and mean intensity were calculated and also observed host species correlation with sex, localities and seasons [21]. To analyse prevalence, mean, abundance and mean intensity by using the Fisher test and difference of proportions, while Bootstrap test 97.5% confidence limit by using Quantitative Parasitology 3.0 software [22].

RESULTS

A total of 40 samples were captured and analysed for the current study: 21 R. rattus and 19 Mus musculus. The two Hymenolepididae species were found in the sub-urban localities of Hyderabad district. These localities are mostly under development, lack freshwater facilities and have sanitary risks. This cestode is found in the small intestine. Morphometric studies were conducted on both species: H. nana and H. diminuta. H. nana has been identified based on 375 samples. These cestode were white creamy. The body of the cestode is thin and long. Body consist on scolex, immature proglottids, and mature proglottids. In this species scolex with armed rostellum has crown shaped 20-22 hooks. Multistrobia broader length than body length. Usually, immature proglottids were short in size. Mature proglottids contain male as well as female reproductive organs. In the segment male reproductive organ has three globular testes, one is polar and two are antipodal. There is a seminal vesicle, one on the outer, and one on the inner side, that opens to cirrus and is surrounded by a cirrus pouch. The female organ ovary is usually bi-lobed. However, in the present specimen, it was not prominent. Ootype was connected with the vitelline duct from the vitelline glands. The gravid segments contain several eggs, each egg has six hooks, the oncosphere is covered with a thin layer called hyaline, and a hard outer layer called filaments (Figure 1).

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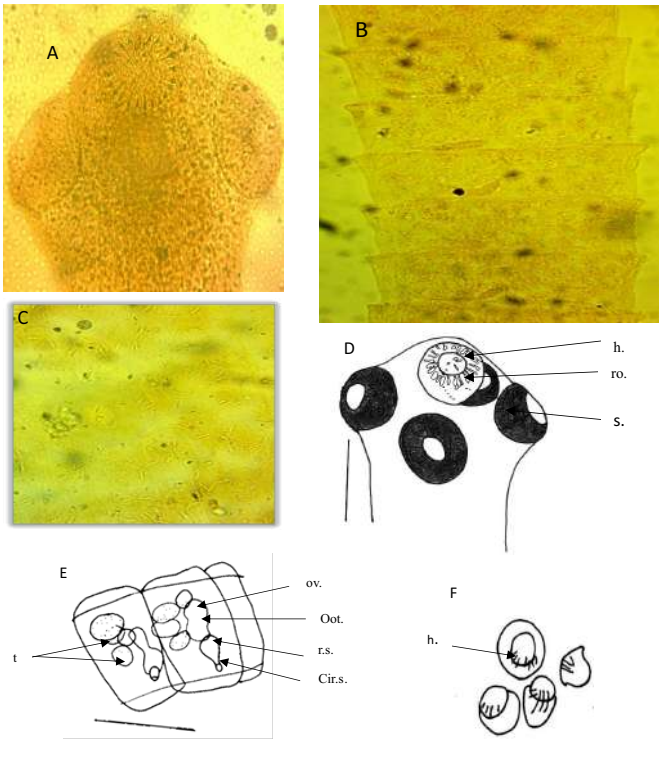

Figure 1: Hymenolepis Nana (Dwarf Tapeworm) A, B, and C Photographs on Omax 40 x2500 Trinocular Compound Microscope with a 10MP camera. D, E and F Line Drawing on Olympus ch.20.(D)Scolex,

h=Hooks, ro=rostellum, s=suckers. (E) Mature segment t=Testis, cir.s.=Cirrus Sac/Pouch, oot. =Öotype, ov. =Ovary, and r. s.=Receptaculum Seminis.(F)Eggs with Hooks. Scale bar 0.1mm.

H. diminuta was identified based on 327 specimens of cestode. Strobila was well- developed,reaching its maximum length. The body is consisting of colex, immature proglottid, and mature proglottid. Scolex was broader, with a non- recognizable neck. Scolex was flattened slightly towards the dorsal and ventral surface. Suckers were round or oval without armed, and located anterolateral. Rostellar pouch was present. Neck was wider, slightly smaller than segment. Mature proglottids were diagonal with quadri-lateral single pairs of parallel sides. Testes were round spherical, small almost equal in size, one was polar and the other two were antipodal. Cirrus sac was bipartite, well developed, one elongated, short and the other part was overly ventral x-axis canal. The genital atrium was situated at lateral side of proglottids, however, it was not fully developed. Ovary was broad, irregularly lobed, and situated at the middle field of the proglottids vitellarium posterior to the ovary, barely lobed. The gravid segment was an elongated fully developed uterus. The uterus contains numerous small eggs. Egg were round, and oncosphere had embryonic hooks(Figure 2).

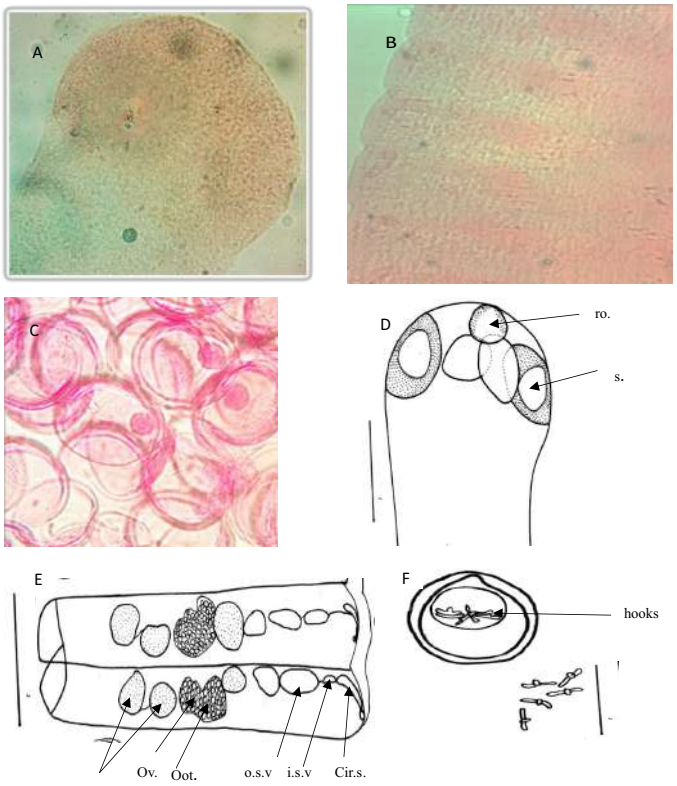

Figure 2: *Hymenolepis diminuta* A, B, and C Photographs on Omax 40 x2500 Trinocular Compound Microscope with a 10MP Camera. D, E and F Line Drawing on Olympus ch.20.(D)Scolex,

Table 1: Biometric Data of *H. nana* and *H. diminuta*

Body Parts	*H. nana*	*H. diminuta*
Scolex	0.0638X0.083	0.236X0.111
Suckers	0.055X0.066	0.097X0.08
Rostellum	0.083X0.611	0.055X0.027
Hooks	0.0166	-
Immature Segment	0.111	0.222
Mature Segment	0.152	2
Testes	0.0277	0.122
Ovary	0.055	0.244
Gravid Segment	0.116	1.777
Eggs	0.027	0.333X0.311
Eggs Hooks	0.0083	0.07

Statistical analysis of rodents revealed that the maximum prevalence of Hymenolepididae species was more frequent in Rattus rattus than in Mus musculus. The maximum mean intensity and mean abundance of H. diminuta was more in Mus musculus, and that of H. nana was more in Rattus rattus. The allocated localities, maximum prevalence, mean intensity and abundance in Qasimabad, followed by Saddar remaining two are minimum prevalent. Moreover, in season prevalence, mean intensity and abundance are maximum in Summer- spring than winter –autumn. However, no significant differences were seen in survey areas, divided years, and host species (Table 2).

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Table 2: Prevalence, Mean Intensity, and Abundance

Host	Statistical Analysis	*H. nana*	*H. diminuta*
Rattus Rattus Linnaeus, (1758) n=21	P(%)	(18) 85.7	(18) 85.7
	Mean Intensity	(255) 14.16	(194) 11
	Mean Abundance	(255) 12.14	(194) 9.8
Mus Musculus Linnaeus, (1758) n=19	P(%)	(15) 78.9%	(15) 78.9%
	Mean Intensity	(120) 8	(233) 15.53
	Mean Abundance	(120) 6.31	(233) 12.26
Areas			
Qasimabad n=10	P(%)	(8) 80	(8) 80
	Mean Intensity	(36) 4.50	(72) 9.50
	Mean Abundance	(36) 3.60	(72) 7.60
Latifabad n=9	P(%)	(5) 55.6	(5) 55.6
	Mean Intensity	(15) 3.00	(15) 6.00
	Mean Abundance	(15) 1.67	(15) 3.33
Sadar n=6	P(%)	(4) 66	(4) 66
	Mean Intensity	(20) 5.00	(30) 7.50
	Mean Abundance	(20) 3.33	(30) 5.00
Hyderabad City n=5	P(%)	(3) 60	(3) 60
	Mean Intensity	(16) 5.33	(16) 5.33
	Mean Abundance	(16) 3.20	(16) 3.20
Season			
Winter –Autumn n=20	P(%)	(8) 40	(8) 40
	Mean Intensity	(36) 4.50	(51) 5.67
	Mean Abundance	(36) 1.80	(51) 2.55
Summer –Spring n=20	P(%)	(16) 80	(16) 80
	Mean Intensity	(158) 9.50	(158) 9.50
	Mean Abundance	(158) 7.60	(158) 7.60

DISCUSSION

The objective of the current research is to study intestinal cestode species in rats and mice. Therefore, we evaluated the prevalence, mean and abundance in urban environments to reach the relevance of rats and mice zoonotic parasites for public health. Rats and mice captured can be separated by location type and season. Most rats and mice were captured from Qasimabad: location type summer and spring seasons. In statistical analysis of the current study, H. nana and H. diminuta species were more prevalent in Rattus rattus than Mus musculus, and significantly higher in Qasimabad than any other location type. Previous studies have reported that H. nana and H. diminuta are zoonotic parasites from zoonotic humans. These cestodes are transmitted to humans by intermediated hosts. Whereas, in humans, it is asymptomatic and causes headache, weakness, stomach ache and sometimes diarrhoea [23]. Veterinary-relevant species H. diminuta, and H. nana have been reported previously in the Netherlands in three areas: Farms, rural and suburban environments. The prevalence of H. diminuta was more in brown rats in suburban areas [24]. In contrast, our study showed that both species were frequently seen in sub-urban rats and mice. It may be due to different hygiene conditions of environments. Endo-parasites are related to human health in rodents: Rattus rattus, Rattus norvegicus, and Mastomys natalensis. A study on the effect of parasites in various aspects such as location, seasons, age of hosts, and gender of hosts was done previously and reported the prevalence of eight species including H. nana (0.8%) and H. diminuta (17.2%) in the metropolitan area of South Africa [25]. These findings are concordant with our findings. Current data revealed that H. nana and H. diminuta are more prevalent in Rattus rattus than in Mus musculus. However, identifying typical cestode of rodents is tricky, especially hymenolepid species because H. nana and H. diminuta act as cryptic species. Furthermore, morphological identification by combining molecular and phylogenetic analysis has been done previously in Egypt, where they found prevalence of H. nana (57%) and H. diminuta (35%) in Rattus rattus in different locations of Egypt Aswan Governorate. Mainain aim of the study was to shed light on zoonotic cestodes for the better health of humans [26]. Rodents-borne cestode especially H. nana has been reported in Aswan School children. These rodents lead to parasitic risk for humans and have zoonotic importance [27-29]. Similarly, in Pakistan, urban localities of Lahore have been studied for H. diminuta in three different host species: Rattus rattus, Rattus norvegicus, and Mus musculus to study the prevalence of H. diminuta in seasons and months. The maximum prevalence of H. diminuta was seen in rat species than in mice [30]. Our study poses that the current findings are important for dynamics, health risk and socio-environmental factors, concerning cestode infection in humans. Taking into account the prevalence of cestode parasites could lessen the burden of zoonotic diseases. Furthermore, measures should be taken to improve the sewage system for better health prospects.

CONCLUSIONS

It was concluded that there were considerable differences between host species and cestode species. Rattus rattus (85%) was infected than Mus musculus (78%). Location-wise, the most prevalent area was Qasmabad. Both cestode species were prevalent in the summer and spring seasons. The rats and mice have been living with humans, therefore, it is an initiative to minimize the population of rodents.

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Authors Contribution

Conceptualization: MR
Methodology: MR, NAB
Formal analysis: MR
Writing review and editing: NAB

All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

All the authors declare no conflict of interest.

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Proximate Composition of *Rita rita* from Southern Punjab, Pakistan

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ABSTRACT

Fish and fish products are considered essential in the human diet due to their high nutritional content, particularly protein and omega-3 fatty acids, which are believed to help maintain good health and prevent cardiovascular, inflammatory, and neurological conditions. **Objective:** To evaluate the proximate *Rita rita* body composition of the freshwater catfish *Rita rita* from Pakistan. **Methods:** Fifty samples of were collected from River Chenab Head Muhammad wala, Multan, Punjab and their proximate body composition was evaluated. Descriptive statistics were used to summarize the data, while independent t-tests and correlation analyses were performed to evaluate relationships among morphometric parameters and body constituents. **Results:** Results showed that the fish contained 77.62 ± 3.47% water, 0.73 ± 0.19% ash (wet weight), 0.87 ± 0.19% fat (wet weight), and 20.78 ± 3.32% protein (wet weight). Correlation analysis revealed that water content was highly significantly correlated with protein (r=0.996), organic content (r=0.999), and ash (r=0.339), while body weight showed significant correlations with most body constituents, including fat (r=0.808) and protein (r=0.628). **Conclusion:** It is concluded that fish collected from the studied sampling site comprises good nutritional quality, especially in respect to fats and protein content, and hence highly recommended for consumption. Findings of the research work will be important for consumers to select proteinaceous fish and useful for nutritionists and ichthyologists working on the fish quality meat.

INTRODUCTION

In aquaculture, growth is a key component characterized by changes in size and tissue composition. Fish and fish products are considered essential in the human diet due to their high nutritional content, particularly protein and omega-3 fatty acids, which are believed to help maintain good health and prevent cardiovascular diseases [1, 2]. Due to their higher content of polyunsaturated fats compared to other animal fats, fish are beneficial in medicine, particularly for lowering blood cholesterol [3]. The proximate composition of each fish species varies and is influenced by both exogenous and endogenous factors, including feeding conditions, water quality, fish sex and age, catch period, water temperature, feeding habits, seasonal variations, species-specific traits, condition factor, size, and activity levels [4-6]. The study of these proximate components provides a clear understanding of the energy value of fishes [7]. Moisture percentage can indicate content, with lower moisture indicating higher level of these content. For instance, different species of fish were reported to have low fat, protein, and calorie protein content in the incidence of high water content of muscle [8]. The study of muscle components of fish such as fat, protein, and calories, gives us a clear understanding in assessing the energy value of the fishes. Fish typically contains 66%–81% water, 16%–21% protein, 1.2%–1.5% mineral, 0.2%–25% fat, and 0%–0.5% carbohydrate. Carbohydrates and non-protein compounds are often in negligible percentages, typically <0.5% [7, 8]. The freshwater catfish, which belongs to the family Bagridae and the genus Rita, is commonly found in South Asia and is

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highly valued for its flavorful meat [9].

This study aimed to evaluate the proximate body composition of the freshwater catfish *Rita rita* from Pakistan.

METHODS

For this study, a total of fifty samples were taken and tested. Fifty samples of *Rita rita* were collected from River Chenab Head Muhammad wala, Multan, Punjab, Pakistan. Each specimen was weighed to the nearest 0.01 gram after debris and water removal. Body length was measured to the nearest 0.01 centimeter. Sample were dried in an oven (70-80°C) until the constant mass was obtained [10]. The total moisture was calculated as

Water content = Initial weight -Weight after drying

The dry substance of each fish specimen was ground and homogenized, followed by storage in airtight plastic jars. Ash content was measured by burning the dry samples in a muffle furnace (550°C for 24 hours). After cooling, the samples were weighed to determine ash content as:

Ash content=Initial weight -Weight loss after incineration

A 2:1 v/v mixture of chloroform and methanol was used to extract fat content [11]. The weighted powder of each fish sample was placed in a test tube, combined with the solvent solution, stirred, and covered with aluminum foil. It was kept overnight and then centrifuged. The clear supernatant was transferred into pre-weighed glass bottles and evaporated in an oven to leave behind lipid fractions. The total fat content was determined by using formulae [10]:

Fat = initial weight of sample – final weight of sample x 100

The protein content was calculated by [12]:

Protein content = 100 – (All Other Body Contents)

The current study utilized the Statistical Package for the Social Sciences software (SPSS) version 26 for all statistical tests. Descriptive analysis was used to find the mean, standard deviation and range of continuous variables. The independent sample t-test analysis was used to explore the difference in the mean body composition parameters between the groups. To assess the nature and the degree of association between two quantitative variables, correlation analysis was conducted with results presented in form of Pearson's correlation coefficients (r). Regression analyses were run for the study looking at the effects of body length and weight on composition parameters Further, standard errors and the R^2 values for each of the developed models are presented. The level of significance used for study was 0.05.

RESULTS

Fifty samples of wild *Rita rita* were selected for estimation of their body composition study. The analysis revealed an average of water composition (%) to be 77.62 ± 3.47 (range: 70.86 to 83.47). Protein content being significantly high was reported to be 20.78±3.32 (range: 15.34 to 27.40) in wet weight and 92.79± 1.33 (range: 90.07 to 95.50) in dry weight. However, fat and ash levels remained comparatively low across both wet and dry weights as shown in table 1.

Table 1: Mean and Range Values of Various Body Constituents of *Rita rita*

Parameter	Mean ± S.D	Range
Water%	77.62 ± 3.47	70.86-83.47
Ash Wet Wt. %	0.73 ± 0.19	0.33-1.15
Ash Dry Wt. %	3.29 ± 1.87	1.87-5.53
Fat Wet Wt. %	0.87 ± 0.54	0.54-1.45
Fat Dry Wt. %	3.92 ± 0.79	2.55-5.73
Protein Wet Wt. %	20.78 ± 3.32	15.34-27.40
Protein Dry Wt. %	92.79 ± 1.33	90.07-95.50
OC Wet Wt. (%)	21.65 ± 3.41	16.08-28.38
OC Dry Wt (%)	96.71 ± 0.81	94.47-98.13

Wet Wt=ash in wet weight; Dry Wt=dry weight; OC Content=Organic content

Statistical analyses of total length and other body constituents in both wet wt. and dry wt. of *Rita rita* respectively (Table 2). Total length showed highly significant with % water with r=0.669, fat wet wt with r value 0.784, percent protein wet wt. r=0.626 and percent organic content in wet wt. r=0.654; significant correlation was found with percent ash wet wt, correlation r=0.485; least significant with percent fat dry wt. as r=0.385 and non significant correlation with percent ash, protein and organic content dry weight.

Table 2: Descriptive Statistical Analysis of Total Length (TL, cm) With Various Body Constituents for *Rita rita*

Equation	a	b	S.E (b)	r	r^2
% Water = a+b TL	27.857	2.511	0.403	0.669***	0.447
% Ash Wet Wt. = a+b TL	2.682	-0.098	0.026	-0.485**	0.236
% Ash Dry Wt. = a+b TL	5.464	-0.109	0.125	$-0.125^{n.s}$	0.016
% Fat Wet Wt. = a+b TL	4.090	-0.162	0.019	-0.784***	0.615
% Fat Dry Wt. = a+b TL	9.984	-0.306	0.115	-0.358*	0.128
% Pro. Wet Wt. = a+b TL	65.370	-2.250	0.405	-0.626***	0.392
% Pro. Dry Wt. = a+b TL	84.552	0.416	0.200	$0.288^{n.s}$	0.083
% OC Wet Wt. = a+b TL	69.460	-2.412	0.403	-0.654***	0.427
% OC Dry Wt. = a+b TL	94.536	0.109	0.125	$0.125^{n.s}$	0.016

Pro= protein; r = Correlation Coefficient; a = Intercept; b = Slope; S.E= Standard Error

Log total length showed highly significant with % water, ash wet wt., fat wet wt., protein wet wt. and organic content wet wt. with r values 0.666, 0.507, 0.782, 0.622 and 0.651 respectively. Significant with fat dry wt. with r=0.369 and all others showed non-significant correlation as shown in table 3.

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Table 3: Descriptive Statistical Analysis of Log Total Length (TL, cm) With Various Body Constituents for *Rita rita*

Equation	a	b	S.E (b)	r	r^2	t-value
Log % Water = a+b Log TL	1.062	0.638	0.103	0.666***	0.443	-22.864
Log % Ash Wet Wt. = a+b Log TL	3.612	-2.902	0.712	-0.507***	0.257	-8.292
Log % Ash Dry Wt. = a+b Log TL	1.424	-0.710	0.771	$-0.132^{n.s}$	0.017	-4.814
Log % Fat Wet Wt. = a+b Log TL	4.774	-3.736	0.429	-0.782***	0.612	-15.693
Log % Fat Dry Wt. = a+b Log TL	2.586	-1.543	0.562	-0.369**	0.136	-8.088
Log % Pro. Wet Wt. = a+b Log TL	4.042	-2.106	0.382	-0.622***	0.387	-13.357
Log % Pro. Wet Wt. = a+b Log TL	1.855	0.087	0.042	$0.283^{n.s}$	0.080	-68.619
Log % OC Wet Wt. = a+b Log TL	4.145	-2.171	0.365	-0.651***	0.424	-14.169
Log % OC Dry Wt. = a+b Log TL	1.958	0.021	0.026	$0.120^{n.s}$	0.014	-116.661

Statistical analyses in table 4. showed highly significant correlation with % water with r=0.679, ash wet wt. with r value 0.611, percent fat wet wt. with r=0.808, percent protein wet and dry wt. r=0.628 and percent organic content in wet wt. r=0.657; significant correlation was found with percent fat dry wt, correlation r=0.375; non-significant correlation with percent ash, and organic content dry weight.

Table 4: Descriptive Statistical Analysis of wet body wt. (g) with various body constituents for *Rita rita*

Equation	a	b	S.E (b)	r	r^2
% Water = a+b TL	62.630	0.183	0.029	0.679***	0.460
% Ash Wet Wt. = a+b TL	1.460	-0.009	0.002	-0.611***	0.373
% Ash Dry Wt. = a+b TL	4.584	-0.016	0.009	$-0.251^{n.s}$	0.063
% Fat Wet Wt. = a+b TL	1.855	-0.012	0.001	-0.808***	0.652
% Fat Dry Wt. = a+b TL	5.803	-0.023	0.008	-0.375**	0.141
% Pro. Wet Wt. = a+b TL	34.054	-0.162	0.029	-0.628***	0.394
% Pro. Dry Wt. = a+b TL	34.054	-0.162	0.029	-0.628***	0.394
% OC Wet Wt. = a+b TL	35.909	-0.174	0.029	-0.657***	0.431
% OC Dry Wt. = a+b TL	95.416	0.016	0.009	$0.251^{n.s}$	0.063

ns= not significant

Statistical analyses of log wet wt. and other body constituents in both wet wt. and dry wt. of *Rita rita* respectively, as shown in table 5. Log wet wt. showed highly significant correlation with % water with r=0.674, ash wet wt. with r value 0.635, percent fat wet wt. with r=0.817, percent protein wet wt. r=0.635 and percent organic content in wet wt. r=0.665; significant correlation was found with percent fat dry wt, and protein dry weight correlation r=0.368; non-significant correlation with percent ash, and organic content dry weight.

Table 5: Descriptive Regression Analysis of Log Wet Body wt. (W, g) with Different Body Constituents for *Rita rita*

Equation	a	b	S.E (b)	r	r^2	t-value
Log % Water = a+b Log TL	1.521	0.193	0.031	0.674***	0.455	-91.887
Log % Ash Wet Wt. = a+b Log TL	1.923	-1.087	0.191	-0.635***	0.404	-21.438
Log % Ash Dry Wt. = a+b Log TL	1.289	-0.411	0.225	$-0.255^{n.s}$	0.065	-15.185
Log % Fat Wet Wt. = a+b Log TL	2.156	-1.166	0.119	-0.817***	0.668	-35.096
Log % Fat Dry Wt. = a+b Log TL	1.522	-0.491	0.166	-0.392**	0.154	-21.011
Log % Pro. Wet Wt. = a+b Log TL	2.537	-0.642	0.113	-0.635***	0.403	-32.287
Log % Pro. Wet Wt. = a+b Log TL	1.903	0.034	0.012	0.368**	0.135	-241.059
Log % OC Wet Wt. = a+b Log TL	2.596	-0.663	0.107	-0.665***	0.443	-34.130
Log % OC Dry Wt. = a+b Log TL	1.961	0.013	0.007	$0.237^{n.s}$	0.056	-399.981

Log WW= Logarithm of Wet Weight

The condition factor showed significant correlation with percent ash dry wt, correlation r=0.388 and non-significant correlation with all remaining body constituents (Table 6).

Table 6: Descriptive Statistical Analysis of Condition Factor with Various Body Constituents for *Rita rita*

Equation	a	b	S.E (b)	r	r^2
% Water = a+b TL	68.908	8.319	6.616	$0.179^{n.s}$	0.032
% Ash Wet Wt. = a+b TL	1.755	-0.978	0.335	-0.388**	0.151
% Ash Dry Wt. = a+b TL	6.561	-3.120	1.500	$-0.288^{n.s}$	0.083
% Fat Wet Wt. = a+b TL	1.558	-0.657	0.359	$-0.255^{n.s}$	0.065
% Fat Dry Wt. = a+b TL	5.312	-1.332	1.519	$-0.126^{n.s}$	0.016
% Pro. Wet Wt. = a+b TL	27.778	-6.685	6.365	$-0.150^{n.s}$	0.022
% Pro. Dry Wt. = a+b TL	88.126	4.452	2.505	$0.249^{n.s}$	0.062
% OC Wet Wt. = a+b TL	29.337	-7.342	6.524	$-0.160^{n.s}$	0.026
% OC Dry Wt. = a+b TL	93.439	3.120	1.500	$0.288^{n.s}$	0.083

Where K is the condition factor

Statistical analyses of log condition factor and other body constituents in both wet wt. and dry wt. of *Rita rita* respectively, as shown in Table 7. Condition factor showed significant correlation with percent ash wet wt, correlation r=0.398, least significant with percent ash dry weight with r=0.303 and non-significant correlation with all remaining body constituents.

Table 7: Descriptive Statistical Analysis of Log Condition Factor with Various Body Constituents for *Rita rita*

Equation	a	b	S.E (b)	r	r^2	t-value
Log % Water = a+b Log TL	1.887	0.108	0.090	$0.171^{n.s}$	0.029	-32.176
Log % Ash Wet Wt. = a+b Log TL	-0.122	-1.501	0.499	-0.398**	0.158	-9.015
Log % Ash Dry Wt. = a+b Log TL	0.525	-1.074	0.488	-0.303*	0.092	-8.343
Log % Fat Wet Wt. = a+b Log TL	-0.055	-0.803	0.439	$-0.255^{n.s}$	0.065	-8.658

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Log % Fat Dry Wt. = a+b Log TL	0.592	-0.376	0.395	-0.136$^{n.s}$	0.019	-8.555
Log % Pro. Wet Wt. = a+b Log TL	1.319	-0.377	0.317	-0.169$^{n.s}$	0.029	-10.644
Log % Pro. Wet Wt. = a+b Log TL	1.966	0.051	0.028	0.250$^{n.s}$	0.063	-104.409
Log % OC Wet Wt. = a+b Log TL	1.338	-0.394	0.312	-0.179$^{n.s}$	0.032	-10.881
Log % OC Dry Wt. = a+b Log TL	1.985	0.033	0.016	0.284$^{n.s}$	0.081	-182.520

Statistical analyses of percent water and log percent water with other body constituents in both wet wt. and dry wt. of *Rita rita* respectively, as shown in Table 8 and 9. Percent water showed highly significant with total length (TL) with r=0.669, with wet wt. r=0.679, fat wet wt with r value 0.502, percent protein wet wt. r=0.996 and percent organic content in wet wt. r=0.999; least significant correlation was found with percent ash wet wt, correlation r=0.339; and non significant correlation with percent ash, fat, protein and organic content in dry wt. of analysis.

Table 8: Descriptive Regression Analysis of Percent Water with Different Body Constituents for *Rita rita*

Equation	a	b	S.E (b)	r	r^2
TL= a+b % water	5.993	0.178	0.029	0.669***	0.447
W = a+b % Water	-113.626	2.521	0.394	0.679***	0.460
% Ash Wet Wt. = a+b % Water	2.154	-0.018	0.007	-0.339*	0.115
% Ash Dry Wt. = a+b % Water	-0.695	0.051	0.033	0.221n.s	0.049
% Fat Wet Wt. = a+b % Water	3.019	-0.028	0.007	-0.502***	0.252
% Fat Dry Wt. = a+b % Water	0.018	0.050	0.032	0.221n.s	0.049
% Pro. Wet Wt. = a+b % Water	94.827	-0.954	0.012	-0.996***	0.993
% Pro. Dry Wt. = a+b % Water	100.677	-0.102	0.054	-0.264n.s	0.070
% OC Wet Wt. = a+b % Water	97.846	-0.982	0.007	-0.999***	0.997
% OC Dry Wt. = a+b % Water	100.695	-0.051	0.033	-0.221n.s	0.049

Table 9: Descriptive Regression Analysis of log % Water with Different Body Constituents for *Rita rita*

Equation	a	b	S.E (b)	r	r^2	t-value
Log TL= a+b Log % Water	-0.015	0.694	0.112	0.666***	0.443	-20.525
Log W = a+b Log % Water	-2.537	2.353	0.372	0.674***	0.455	-1.740
Log % Ash Wet Wt. = a+b Log % Water	3.793	-2.087	0.807	-0.350**	0.122	-6.305
Log % Ash Dry Wt. = a+b Log % Water	-2.012	1.332	0.788	0.237$^{n.s}$	0.056	-2.118
Log % Fat Wet Wt. = a+b Log % Water	4.747	-2.549	0.617	-0.512***	0.262	-8.988
Log % Fat Dry Wt. = a+b Log % Water	-1.058	0.869	0.618	0.199$^{n.s}$	0.040	-3.450
Log % Pro. Wet Wt. = a+b Log % Water	7.935	-3.505	0.059	-0.993***	0.987	-110.892
Log % Pro. Wet Wt. = a+b Log % Water	2.130	-0.086	0.044	-0.269$^{n.s}$	0.072	-69.403
Log % OC Wet Wt. = a+b Log % Water	7.870	-3.461	0.046	-0.996***	0.992	-140.967
Log % OC Dry Wt. = a+b Log % Water	2.065	-0.042	0.026	-0.228$^{n.s}$	0.052	-116.483

DISCUSSION

Present study describes comprehensive information and scientific evidence of proximate composition of freshwater edible fish of *Rita rita*. Water content in the present study was found in normal range (60-80%) as reported by various scientists. Percentage of this content was found very close to that of farmed *Ctenopharyngodonidella* (80.76 ± 4.40) reported by Khalid and Naeem [13] in Hybrid , (79.22- 80.83%) reported by Iqbal *et al.* [14]; and in the head flesh of *Rita rita* (84.5%) as studied by Khan *et al.*, [9]. However, Gul *et al.* have found lower percentage of this content in some species (*Garragotyla*, *Garragotyla*) of family Cyprinidae [15]. Ash content range in fish is reported as 0.89-8.00% in different fish species by various fisheries scientists. This constituents in *Rita rita* in the present work being 2.77% shows to be in normal limit. Percent value of ash was found comparable with that of studied by Naeem *et al.* in *Cirrhinus mrigala* (2.87±0.54%) [14], by Kousar *et al.* in Genetically Improved Farmed Tilapia (GIFT) (2.75- 3.30%) [16]. Another study also found low percentage of ash (0.89%) in the flesh of *Rita rita*. Our findings are however opposed by the results that reported too high percentage (8.00%) of this content in another Cyprinid [15]. Fat contents of *Rita rita* were found to be only 2.98% for the present study and within the range (2.5-6.00%) as studied by various ichthyologists. This finding is in agreement with the results that highlight the fat content of Puntius chola and *Cirrhinus mrigala*, respectively [9]. However, on the contrary, a previous study done on seven fishes reported the maximum lipid and fat content in *Rita rita* [17]. Comparison of protein content among different fish species shows that it ranges from 10-20% in wet wt. of fish. Hence, this constituent was found within the range as documented by various authors. Bano *et al.*, have also documented percentage of protein range in *Labeocalbasu* being 13.87- 15.66% in different treatments feeding different levels of dietary protein [18]. This study also depicted the effect of fish size on the body composition of *Rita rita*. Previously, Mitra *et al.*, also determines the impact of size on the biochemical composition of *Rita rita* fish, showing the medium sized fishes with highest protein and minimum fats [19]. These finding also verifies the results of different studies also documented a definite effect of size of *Ctenopharyngodon idella*, *Catla catla*, *Labeoo calbasu*, GIFT (Genetically Improved Farmed Tilapia), *Clarias batrachus* and Hybrid fish (*Catlacatla*♂ and *Labeorohita*♀) on proximate composition [13, 16, 20]. Though, in spite of the differences, the range of protein in different species of fish study shows that these fishes are good sources of protein to consumers. Present

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study describes comprehensive information and scientific evidence of proximate composition of freshwater edible fish of *Rita rita*. The proximate composition of *Rita rita* was evaluated to determine its importance and quality for human consumption. Results of the present study indicated that *Rita rita* constitutes a low fatty acid and ash content while a high source of protein and thus can be described as an ideal dietetic fish food for human consumption.

CONCLUSIONS

This study highlights the proximate composition of *Rita rita*, indicating its quality in terms of its nutritional value, high protein content and low amounts of fat. The correlations between morphometric parameters and body constituents imply that size and condition enhance the nutritional value of the fish. Thus, these results support the inclusion of *Rita Rita* in the diet and may interest people who consciously care about their diet. This research contributes new information to ichthyologists and nutritionists to encourage the consumption of *Rita rita* in human diets. Enhancing the nutritional parameters of this dish is also an aspect that further research should explore.

Authors Contribution

Conceptualization: NB, MAR
Methodology: SUR
Formal analysis: SUR
Writing, review and editing: SUR, HMI

All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

All the authors declare no conflict of interest.

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Infestation of Helminth Parasites in Goat and Sheep in Tehsil Charbagh at District Swat

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ABSTRACT

Many rural people rely primarily on small ruminants like sheep and goats as a source of income, particularly in dry and semi-arid areas. However, the health and productivity of livestock are greatly impacted by gastrointestinal helminth infections, which can result in major financial losses. **Objective:** To assess the prevalence and different types of gastrointestinal helminth parasites among sheep and goats at Tehsil Charbagh, District Swat. **Methods:** Faecal samples were collected from eighty sheep and seventy goats. The samples were stored in sealed containers at 4°C until they were analyzed. To determine whether helminth eggs were present, standard flotation procedures were used. **Results:** Helminth parasites were detected in 105 (70%) of the total samples. In sheep, the prevalence was 51.43%, whereas in goats, it was 48.57%. The identified helminth parasites were *Trichuris vulpis*, *Nematodirus spathiger*, *Haemonchus contortus* and *Trichostrongylus axei*. The species-wise incidence in goats was 1.96% for *N. spathiger* and *T. axei*, 31.37% for *T. vulpis*, and 64.70% for *H. contortus*. In sheep, the incidences of *T. Vulpis* and *H. contortus* were 72.22% and 27.78%, respectively. **Conclusions:** The high incidence of helminth infections in goats and sheep suggests that the health of the livestock in the research area is seriously threatened. These results highlight the necessity for systematic deworming procedures, expanded epidemiological research, and more awareness to promote sustainable management of livestock and enhance rural livelihoods.

INTRODUCTION

In Pakistan, livestock production is essential to the livelihood of farmers with limited resources and makes a substantial contribution to rural development, revenue generation, and food security. Approximately 11.6% of the national GDP and 55.1% of the value of agriculture added are attributed to the livestock industry [1]. Sheep, goats, and other small ruminants are important livestock, especially in arid and semi-arid areas. They play social and religious responsibilities, deliver meat, milk, fibers such as wool skin, and carcasses, and provide as an alternative source of emergency cash [2]. One of the most prevalent and dangerous parasites infecting small ruminants is intestinal helminths [3]. These multicellular eukaryotic invertebrates, which mostly live in their hosts' gastrointestinal tracts, comprise nematodes (roundworms), the cestodes (tapeworms), as well as trematodes (flukes) [4]. In goats and sheep, the illnesses collectively referred to as helminthiasis can result in severe morbidity and mortality, which can include diarrhoea, anorexia, decreased milk production, poor weight gain, and finally death [5, 6]. *Trichuris vulpis*, a whipworm that is usually present in the large intestine, is one of the main helminth parasites. Despite being more prevalent in dogs, it has been documented to occur in ruminants and is linked to inflammation, anaemia, and impaired nutritional absorption as a result of mucosal injury [7]. *Trichostrongylus axei*, *Haemonchus contortus* and *Nematodirus spathiger* are other important nematodes

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that lower animal productivity and cause financial losses [8]. Due to decreased fertility, slower development rates, less milk along with wool production, higher treatment costs, as well as mortality, especially in seriously infected animals, helminth infections result in significant economic losses [9]. Environmental factors (temperature, humidity, or rainfall), animal grazing practices, pasture quality and host immunity are some of the factors that affect infection rates [10, 7]. Control becomes more difficult in areas with warm, humid climates because these conditions encourage the growth and survival of the parasite larvae. High intestinal helminth infection rates in small ruminants have been reported by studies carried out in different regions of Pakistan, such as Dir and Peshawar. This study investigated the diversity, prevalence, and associated risk factors of gastrointestinal parasites in both wild and domestic animals across various regions of Pakistan [11]. Studies conducted in many parts of Pakistan, including Dir and Peshawar, have found high gastrointestinal helminth infection rates among small ruminants. Although helminthiasis has been extensively investigated in some ecological areas, few data are reported from Tehsil Charbagh, District Swat location with communal grazing and favorable environmental conditions for parasite transmission. Due to the debilitating effect of helminth infection on animal productivity and health, parasitological surveys have to be carried out at a regional level. The current research concerns identification and determination of the gastrointestinal helminth parasites of the sheep and goats at Tehsil Charbagh, District Swat.

The results of this research can be used in planning local livestock husbandry practices and to direct parasite control measures to enhance animal health and rural livelihood.

METHODS

In total, 150 fecal samples were collected from sheep (n=80) and goats (n=70) across different localities in Tehsil Charbagh District Swat using stratified random sampling. Localities were first identified as strata, and within each stratum, animals were selected systematically (e.g., every 5th animal) or using a random number generator to ensure representativeness. The sample size was determined using the formula $n = (Z^2 \times P(1-P))/E^2$, where Z=1.96 (95% confidence level), P=0.7 (expected prevalence based on regional studies [17,25]), and E=0.1 (10% margin of error). This yielded a minimum requirement of 81 animals per group; our study included 80 sheep and 70 goats, ensuring robust prevalence estimates. Fresh fecal samples of about 10–15 grams. Localities were first identified as strata, and within each stratum, animals were selected systematically (e.g., every 5th animal) or using a random number generator to ensure unbiased representation. Fresh fecal samples of about 10–15 grams per animal were collected directly from the rectum under strict standards, with the immediate use of disposable plastic gloves. Each sample was placed into sterile labeled zip-lock bags along with animal ID, date, and time of collection; samples were transported in a cool box without air and stored at 4°C until laboratory analysis [12]. The flotation method described by Zajac and Conboy (2012) was used to examine samples for helminth eggs. Approximately 3 grams of feces were thoroughly mixed with 42 mL of a 33% zinc sulfate solution (prepared by dissolving 331 g of zinc sulfate in 900 mL of warm distilled water) using a mortar and pestle. The suspension was filtered through a tea strainer to remove debris, and the filtrate was transferred into 15 mL plastic centrifuge tubes [13]. The tubes were centrifuged at 1400 rpm for 4 minutes. A coverslip was gently placed onto each tube and left for 20 minutes to allow egg attachment before being carefully lifted and placed onto a clean glass slide. Slides were examined under a light microscope at 4X and 10X magnification for helminth eggs. Parasite identification was based on the morphological traits of the eggs (size, shape, shell thickness, and internal structures), compared with standard parasitological keys. Descriptive statistics were used to calculate the prevalence of intestinal helminth infestations in goats and sheep. The Chi-square (χ^2) test was applied to compare infection rates between species, and 95% confidence intervals (CI) were calculated to assess the reliability of prevalence estimates. All statistical analyses were performed using IBM SPSS (Version 2021)."

RESULTS

This descriptive study of a cross-sectional nature compared 150 fecal samples (70 sheep and 80 goats) to find the prevalence of gastrointestinal helminth infections in Tehsil Charbagh, District Swat. Of the 105 samples (70%), all were positive for one or more helminth species (Figure 1).

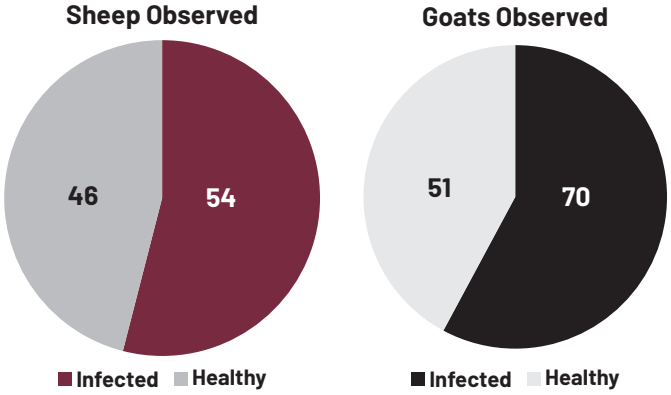

Figure 1: Observed Goats and Sheeps

Incidence of Helminth Infections

Of the 105 exposed animals, 54 (67.5%) were sheep and 51 (72.85%) goats. Infection rates were slightly higher among

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goats, but this did not differ significantly ($\chi^2 = 0.53$, $p = 0.47$), indicating similarly high exposure among species (Table 1).

Table 1: Total Observed Goats and Sheeps

Species	Examined	Infected Frequency (%)
Sheep	80	54 (67.50)
Goat	70	51 (72.85)
Total	150	105 (70.00)

Single vs. Multiple Infections

Single and multiple infections were identified. Multiple infections with helminths were more prevalent, where 44 sheep (81.5% of infected sheep) and 41 goats (80.4% of infected goats) were infected with more than one species. Prevalence of multiple infections was statistically significant ($p < 0.05$), reflecting high environmental exposure to broad range of helminths (Table 2).

Table 2: Single vs. Multiple Infections in sheep and goats

Species	Single Infections	Multiple Infections	Total Infected
Sheep	10	44	54
Goat	10	41	51

Species-wise Distribution of Helminths

Four gastrointestinal helminth species were identified:
Haemonchus contortus (Figure 2)
Trichostrongylus axei (Figure 3)
Nematodirus spathiger (Figure 4)
Trichuris vulpis (Figure 5).

In goats, *H. contortus* was the most prevalent (64.70%), followed by *T. vulpis* (31.37%), and *T. axei* and *N. spathiger* in 1.96% of infected goats (Table 3).

Table 3: Prevalence of Helminths in Goats (n = 51 infected)

Helminth Species	Number Infected Frequency (%)
Haemonchus contortus	33 (64.70)
Trichuris vulpis	16 (31.37)
Trichostrongylus axei	1 (1.96)
Nematodirus spathiger	1 (1.96)

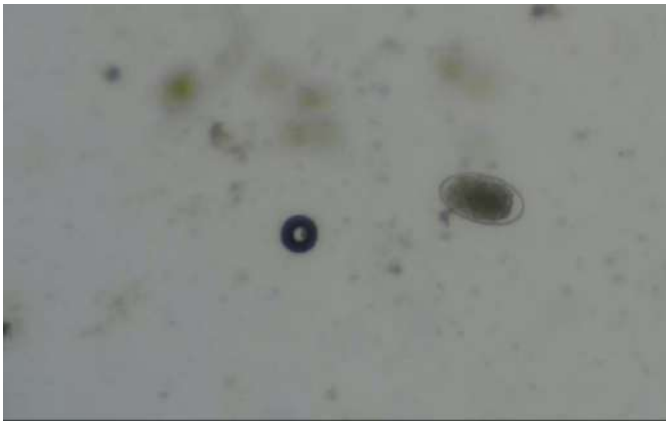

Figure 2: *Haemonchus contortus*

Figure 3: *Trichostrongylus axei*

Figure 4: *Nematodirus spathiger*

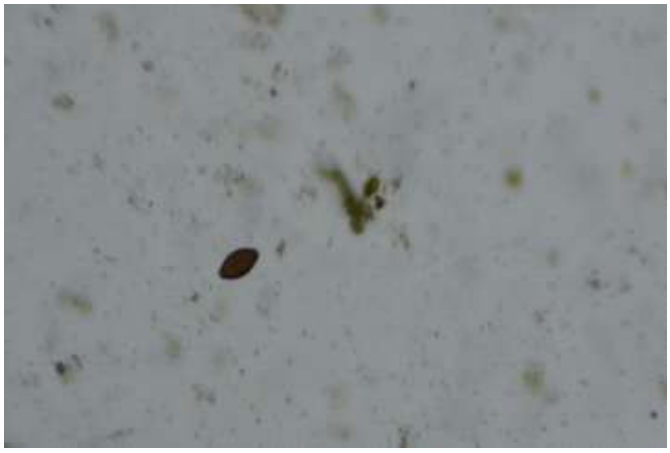

Figure 5: *Trichuris vulpis*

Additionally, *H. contortus* dominated sheep (72.22%), with *T. vulpis* coming in next (27.78%). In sheep, no additional helminth species were found (Table 4).

Table 4: Prevalence of helminths in sheep (n = 54 infected)

Helminth Species	Number Infected
Haemonchus contortus	39 (72.22)
Trichuris vulpis	15 (27.78)

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4. Environmental Factors

It was noted that the prevalence of infections was considerably higher in animals within open grazing areas, particularly where communal pastures and poor sanitation were noted. Specific measurements of environmental conditions (temperature, rainfall, etc.) were hardly taken into record, but from qualitative field observation, many of the positive cases went together with low-lying humid poorly drained grazing conditions-a requirement for larval development and survival.

- Total prevalence: 70% (95% CI: 62.1–77.1%)
- There is no difference in prevalence between sheep and goats ($p = 0.47$)
- Infection rates are greatly impacted by age and grazing system ($p < 0.05$)
- No sexual difference ($p = 0.30$).

DISCUSSION

According to the current study, the overall parasite occurrence in sheep and goat was 67.50% (n=54/80) and 72.85% (n=51/70). Various studies has been published on the prevalence of helminth parasites in sheep and goat. The present study was conducted in Tehsil Charbagh at District Swat. This meta-analysis explores the extent, patterns, and health impacts of helminth polyparasitism in human populations worldwide [14]. This systematic review summarizes the prevalence and types of gastrointestinal helminths affecting domestic ruminants in Ethiopia [15]. This study examined the prevalence of gastrointestinal helminths in dogs on sheep and goat farms in Greece, including the role of wild canids and anthelmintic use [16]. The study of Ashraf *et al.,* conducted in 2022 estimated a general helminth parasite prevalence of 89.20% (281 out of 315 animals) in Upper Dir, Pakistan, with infection rates in sheep 94% (173/184) and in goats 82.43% (108/131). The most prevalent parasites in sheep were *Fasciola hepatica* (13.58%), *Haemonchus contortus* (21.73%), the Trichuris ovis (17.39%), and *Strongyloides papillosus* (41.30%). The frequency of *Trichuris ovis* was 25.20%, Fasciola hepatica was 10.68%, *Haemonchus contortus* was 28.70%, and *Strongyloides papillosus* was 33.33% in goats [16]. In the present study's overall infection rate was 70.0%, the most common genera were *H. contortus, T. vulpis, Nematodirus spathiger,* and *Trichostrongylus axei.* While in May/June and August/September of 2002, the Khan A *et al*. study revealed 36% and 52% infection [17]. The presence of various climatic or environmental factors that could support the survival and development of the infective larval stage of most nematodes could account for the difference in nematode parasites. Nematodes were shown to be equally common in sheep and goats, according to research conducted by Khan R *et al.,* in 2019 [18]. According to the work done by Krishnamoorthy P *et al.*, in 2019, also explained the idea of infection caused by nematodes parasite in small ruminants and elaborate the animal-parasite-vegetation relationship and to use this specific strategy for the control of these harmful nematodes. They also extravagant the idea of disease resistance in many nematodes which have greater influence on their environmental conditions of specific area. They also elucidated the concept that warm and humid environmental conditions of tropical and subtropical regions of the world are best places for the nematodes survival [19]. Which is similar to the current results. However, while in the current investigation only found a 100% overall prevalence rate of nematodes. Variations in the weather and atmospheric humidity may be the cause of the discrepancy in the outcome. While a study conducted on goats in Lahore, Pakistan, found that nematodes had the highest infection rate (42.67%), followed by trematodes (16.67%) and cestodes (4%) while the current study showed that the most common helminth parasites in goats are *Trichuris vulpis, Haemonchus contortus, Nematodirus spathiger* and *Trichostrongyloids axei.* While the most frequent gastrointestinal parasites found in goats and sheep were *Emeria, Trichostrongylus, Haemonchus, Moniezia,* and *Fasciola,* according to Maurizio A *et al.,* in 2023 [20]. According to work done in Lahore region by Mohamed HI *et al.,* in 2021, on different genera of helminths parasites. They have collected 160 fecal samples from different places of the study area. To determine the occurrence of parasites in varios genera, whole samples were exposed to parasitological examination and investigated using the direct smear method [21]. In general, an inclusive occurrence of 40% was verified which exposed 64 samples were positive. The detected parasitic species were *Balantidium coli, Ostertagia ostertagi, Fasciola hepatica, Coccidia, Chabertia ovina, Shistosoma bovis, Trichuris globulosa, Haemonchus contortus,* and *Strogyloides papillosus.* Out of 160 total samples, 64 samples were positive, while 40% prevalence was verified in all ruminants. Among bovines (cows and buffaloes), the multi-parasites prevalence was recorded to be 47.5. However, in ovine (sheep) and caprine (goats), the prevalence was 42.5 and 32.5%, respectively. The parasitic prevalence was observed alike in adults and young. However, the current investigation revealed that *Haemonchus contortus* had the highest occurrence rate, followed by *Trichuris vulpis* in goat and sheep. There was some commonality between the four nematode species that were found in this investigation. While according to Mekonnen, 2007, in goats and sheep of eastern Ethiopia found that Haemonchus had the highest occurrence rate, followed by Trichostrongylus. There were two distinct rainy seasons when the worm burden peaked, between May and

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September. There have been reports of four kinds of flukes and thirteen species of nematodes by Khan W *et al.*, in 2023 [22]. Similar work to our results has also been done by Khan *et al.*, in 2025, in Dir region of Khyber Pakhtunkhwa. Gloved fingers were used to irregularly collect a fecal sample from the rectum of sheep (Ovis aries). The fresh fecal ingredients were placed in sterile plastic vessels with 10% formalin. A total of 584 sheep fecal samples were assembled, and their parasite contents were scrutinized. There were 365 female sheep and 219 male sheep among them. The overall prevalence rate was 89.09%. *Haemonchus species, Strongyloides species, Trichuris species, Fasciola hepaticas species,* and Moniezia species were the most widespread parasites, with relative prevalences of 43.27, 28.57, 15.59, 3.6, and 1.7%. The total incidence of gastrointestinal parasites in male sheep (33.39%) and female sheep (55.65%) differed significantly ($p<0.05$) according to sex. Associated with young lambs (19.86%), the prevalence of gastrointestinal parasites was complex in adult sheep (69.18%). Linked to the Lokhi breed (18.32%), the uppermost infection rates were perceived in the Balkhi breed (38.7%) and the Damani breed (32.53%). In distinction, tehsil Samar Bagh had the maximum frequency of gastrointestinal parasites (17.46%) in the tehsil-wise evaluation, after Samarbagh followed by tehsil Munda (15.23%), Lal Qila (11.01%), Balambat (9.1%), and Khall (8.4%). In the current investigation, sheep and goats had double parasite infection rates of 44 (41.90%) and 61 (58.10%), respectively. Comparably, research from throughout the globe has shown that the current study's findings are very comparable to those of a study carried out by Rafi U *et al.*, 2023 [23]. According to their analysis, the prevalence of infections in sheep and goats was 53.33% and 66.45%, respectively, with the majority of the sample showing multiple infections rather than a single infection in 38 (42.22%) and 156 (50.32) sheep and goats, respectively while the current syudy revealed that 4 species of nematodes were identified which were *H. contortus, T. vulpis, T. axei,* and *N. spathiger.* Among nematodes 6 species were identified which are *H. contortus, T. vitrinus, Strongyloides papillosus, N. spathiger, Ostertagia spp.* and *Trichuri spp.* The similar study was conducted in the Metro Livestock Office in Boalia, Rajshahi District. The study was carried out for 22 months to investigate the prevalence of gastrointestinal helminths parasites in sheep and goat. A total of 240 animals were used in the research. Under a semi-intensive system, the general prevalence of helminthes invasion was found to be 70% (168). Invasions of the trematodal parasites *Fasciola* and *Paramphistomum* were distinguished in goats 21.29% and 18.06% and in cattle 37.64% and 20%, respectively. Invasions of Ascaris and Trichuris nematodes were identified in cattle at 12.94% and 3.52%, and in goats at 25.16% and 3.22%, correspondingly. Goats had a higher frequency of parasitic occurrence (43.75%) than cattle (26.25%). Cross-bred cattle had a larger occurrence of helminths parasites (60.31%) than local-bred cattle (39.68%), but Black Bengal goats (45.71%) were less liable to intestinal parasite contamination than Jamunapari goats (54.28%). According to this study, to minimize parasitic infestation in cattle, proper management, better hygiene, and routine deworming should be ranked. As a result, the conclusions of this study will support researchers and veterinary professionals in working gastrointestinal parasite infestations in this region by Sebatjane PN *et al.*, in 2021 [24]. Their results were similar to the current work. The findings of this study align with previous research indicating a high prevalence of gastrointestinal helminths in small ruminants, particularly in rural and semi-arid regions. Similar results were observed in An-Lemo, Southern Ethiopia, where Sebro *et al.*, reported a significant burden of helminth infections among sheep and goats [25]. The persistence of such infections is often attributed to inadequate deworming practices, lack of awareness, and environmental factors conducive to parasite transmission. Classical literature by Soulsby and contemporary parasitology manuals such as Zajac and Conboy have long emphasized the pathogenic significance and complex life cycles of helminths in domesticated animals, underlining the need for regular surveillance and targeted control strategies. These sources collectively reinforce the urgent requirement for integrated parasite management and routine veterinary intervention to safeguard livestock health and productivity in endemic areas [26, 27].

CONCLUSIONS

According to the current study, sheep had an overall helminth parasite prevalence of 67.50%, whereas goats had a prevalence of 72.85%. *Haemonchus contortus, Trichuris vulpis, Nematodirus spathiger,* and *Trichostrongylus axei* were among the gastrointestinal helminths that were found. The most common species among these were *H. contortus* and *T. vulpis,* suggesting a high parasite burden in the area. On the other hand, the least common species found were *T. axei* and *N. spathiger.* In order to reduce helminthic infections in small ruminants, these findings emphasise the necessity of routine deworming, better pasture management, and more knowledge among livestock owners.

Authors Contribution

Conceptualization: NU
Methodology: SI, HUR, TUR, AR
Formal analysis: SI, HUR, TUR, AR
Writing, review and editing: S, SI, HUR, TUR, AR, NU

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All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

All the authors declare no conflict of interest.

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Impact of Land Use Gradient on Bird Community Structure and Diversity at University of the Punjab, Quaid-e-Azam Campus, Lahore

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ABSTRACT

Growth and development in cities and alterations in land use have a considerable impact on the avian diversity in urbanized landscapes. **Objectives:** To assess current avian diversity, seasonal variation, and long-term changes (1997–2023) in relation to land-use modification within the campus. **Methods:** Fortnightly bird surveys were conducted from May 2022 to April 2023 using the 10-minute point count method at 40 fixed stations representing urban, agricultural, botanical, and wetland habitats. Diversity indices, including the Shannon–Wiener index, species richness, and evenness, were calculated. The methodologies and spatial coverage of these historical studies were not identical to the current survey, and some historical records lacked fine-scale spatial or seasonal data, which is a recognized limitation for direct comparison. **Results:** There were 64 species of birds of 16 orders and 34 families, 40 resident, 10 summer breeding, 8 winter visitor, 5 passage migrant, and 1 vagrant species. The dominance was Passeriformes (63%), non-passerines (37%). The scavenger and generalist birds Corvus splendens, Milvus migrans, and Acridotheres tristis formed more than half the total population. Comparison of trends over a long period revealed a decreasing common myna population and a house crow population, no changes in black kite population, and an increasing prevalence of plain-leaf warbler, yellow-footed green pigeon and cattle egret. Since 2012, urbanization has decreased agricultural habitats and homogenized bird communities. **Conclusions:** Urbanization and the environment of littering have altered the avian diversity, thus the necessity to incorporate biodiversity preservation into urban and campus landscape designs.

INTRODUCTION

The avian population in Pakistan is high and has been reported to comprise 729 species of birds in the land [1]. This is due to its geographical position at the border of the Oriental, Palearctic and Ethiopian zoogeographical regions giving it the importance of endemism in the conservation of the avian biodiversity [2]. Birds are regarded as significant components of the ecosystems since they are signs of the well-being of the environment and play a vital role in the ecosystem, including pollination, seed dispersal, and natural control of pests [3, 4]. The development of lands and human activities like urbanization, agricultural practices, forest destruction and mining have resulted to a number of adverse consequences on the bird population in the globe [5, 6]. Previous research has been conducted to investigate the distribution, migration, and habitat preference of the avian in Pakistan [7, 8]. The studies on natural reserves, wetlands, and farmland in Punjab have evaluated the structure of bird communities and ecological value [9, 10]. Lahore, which is one of the largest urban areas, is studied as well, changing avifauna and the impact of urban growth [11, 12]. Lahore is a campus of the University of the Punjab, which has undergone massive

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urbanization. The buildings, roads, and infrastructure have substituted the previous natural landscape. This notwithstanding, the region has biological value because of its diverse habitats, such as agricultural lands, wastewater pools, botanical gardens, rose gardens, and roadside plants, which favor birds, small mammals, and reptiles [13, 14]. Nevertheless, there is a lack of long-term monitoring. Knowledge of how birds react to the continued urbanization is essential in biodiversity management [15].

The study aims to give a comparative and modern evaluation of avian diversity (2022-2023) over ten years later, comparing long-term changes (1997-2023) and comparing the effect of land-use change on the richness of species and the composition of the species community in the campus.

METHODS

This was a descriptive cross-sectional study conducted to assess the avian community structure and diversity. A systematic sampling technique was employed, with 40 fixed survey stations strategically placed across the major habitat types (urban, agricultural, botanical, and wetland) within the campus. The number of survey stations (n=40) was determined to ensure comprehensive coverage of the major habitat types (urban, agricultural, botanical, and wetland) within the University of the Punjab, Quaid-e-Azam Campus, Lahore. The stations were distributed proportionally to the area of each habitat type. This is the common method in descriptive ecological census whose emphasis is on representative spatial coverage rather than on formal power calculation. The campus is separated into two different environmental zones, an urban habitat and an agricultural habitat. The urban area (387 ha) has the following in it, teaching departments, hostels, administrative and residential quarters, paved ways, and green belts. Comparatively, the agricultural (284 ha) area includes cultivated agricultural land, botanical garden, and ornamental plants cultivation areas as well as two wastewater management ponds amounting to 2.6 ha. The main sources of wastewater that is collected and treated in these ponds are those produced on campus. Site 1 Teaching Departments (258.5 ha); Site 2 Residential Colony and Hostels (129 ha); Site 3 Cultivated Land with wastewater ponds (264 ha); Site 4 Botanical Garden (20.23 ha). The survey of birds was conducted every fortnight between May 2022 and April 2023. During dawn and dusk (summer: 05:0009:00 and 17:0020:00; and winter: 07:0010:00 and 15:3018:00) which were the times of the greatest avian activity they were observed. The identification of the species and geolocation were carried out with the help of binoculars (10x50) and GPS respectively. Standard field guides helped in identification. Being a descriptive cross-sectional study, the analysis was aimed at showing these indices, frequencies, and percentages to describe and compare the avian communities of the two landscapes. Significant inferential statistical tests were not utilized because the purpose of the study was to explain the current community structures and not to test a hypothesis that had been determined beforehand. Five trained observers took part in the survey. To ensure reduced observer variability, two weeks of calibration were carried out prior to data collection where all the participants performed the joint observations in order to harmonize the data collection methods, species recognition, estimation of distance, and counting techniques. This standardization guaranteed the inter-observer reliability during the survey interval. The abundance and diversity of birds were determined by the 10-minute point count technique. The radius of every survey station was 100 meters and the spacing between the stations was a minimum of 500 meters to avoid recording the same individual in two stations. There were 40 survey stations that were distributed randomly in all habitats, which should be urban, agricultural, botanical, and wetlands. All the birds recorded at each station were classified according to the type of habitat and their behavior. The spatial mapping and sampling repeated in various seasons were noted by record of the GPS coordinate of each station. A relative abundance index determined the local occurrence status of the bird's species consisting of the percentage of the number of a given species of birds to the overall number of birds that were seen. To prevent the influence of raw counts, classification was done using relative abundance thresholds. Species that had a percentage of more than 10 of the total individuals were considered as very abundant, those with percentage of 5-10 as abundant, 2-5 as common, 1- 2 as occasional and those with less than 1 percent were considered rare or vagrant. This relative method offers a standard level of comparison of abundance among the habitats and seasons. IBM SPSS version 25.0 was used to analyze all the data (Version 20). Biodiversity indices (such as species richness (S), ShannonWiener diversity index (H7), species evenness (E), relative abundance (pi), and census index (CI)) were calculated based on conventional ecological procedures.

RESULTS

A total of 64 bird species belonging to 16 orders and 34 families were recorded at the Quaid-e-Azam Campus, University of the Punjab, Lahore, from May 2022 to April 2023. Out of these, 40 species were residents, 8 were winter visitors, 10 were summer breeders, 5 were passage migrants, and 1 was an accidental vagrant. The order Passeriformes was dominant, comprising 63% of the total avifauna, while non-passerines represented 37% (Figure 1).

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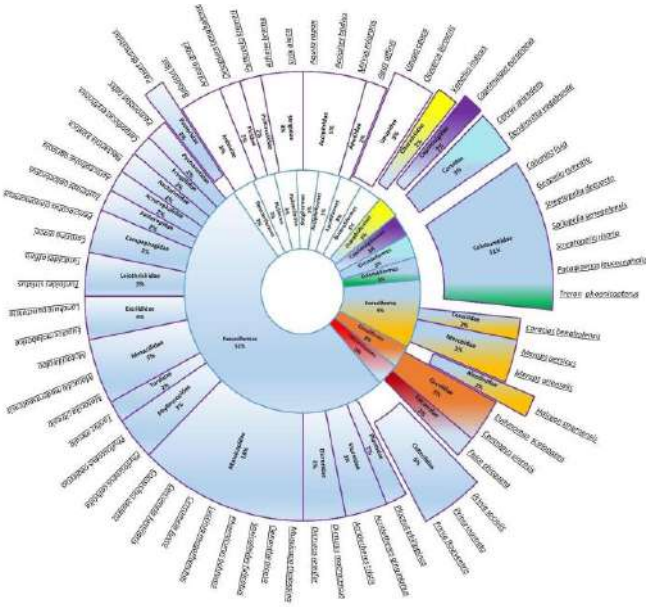

Figure 1: Avian Fauna in Punjab University Based on Taxonomic Distribution

Trends in Urban Bird Populations included Teaching Departments (Site 1): During the summer season, 1,813 individuals of 34 species were recorded. The most dominant species were house crow (Corvus splendens) (Pi=0.267), black kite (Milvus migrans) (Pi=0.161), and common myna (Acridotheres tristis) (Pi=0.111). In winter, 2,060 birds of 42 species were observed, with common crow (Pi=0.183), common myna (Pi=0.156), and black kite (Pi=0.138) as the most abundant. During spring, 2,002, individuals of 42 species were recorded; common crow (Pi=0.188), black kite (Pi=0.175), and blue rock pigeon (Columba livia) (Pi=0.132) dominated. The least observed species across seasons included rufous treepie, Asian koel, and variable wheatear. Residential Area (Site 2): In summer, 1,752 individuals of 34 species were recorded, with common crow (Pi=0.258), common myna (Pi=0.210), and black kite (Pi=0.170) as dominant. During winter, 1,397 individuals of 37 species were observed; black kite (Pi=0.208) and common crow (Pi=0.160) were the most abundant. In spring, 1,424 individuals of 39 species were sighted, dominated by black kite (Pi=0.209) and common crow (Pi=0.206). The least observed species included the Indian robin, verditer flycatcher, and variable wheatear (Table 1).

Table 1: Seasonal Variation in Bird Populations at Urban Sites

Site	Season	Total Individuals	Total Species	Dominant Species (Pi)	Least Observed Species
Teaching Departments (Site 1)	Summer	1,813	34	House Crow (Corvus Splendens) (0.267), Black Kite (Milvus Migrans) (0.161), Common Myna (Acridotheres Tristis) (0.111)	Rufous treepie, Asian koel, Variable wheatear
	Winter	2,060	42	Common Crow (0.183), Common Myna (0.156), Black Kite (0.138)	–
	Spring	2,002	42	Common Crow (0.188), Black Kite (0.175), Blue Rock Pigeon (Columba Livia) (0.132)	Rufous treepie, Asian koel, Variable wheatear
Residential Area (Site 2)	Summer	1,752	34	Common Crow (0.258), Common Myna (0.210), Black Kite (0.170)	Indian robin, Verditer flycatcher, Variable wheatear
	Winter	1,397	37	Black Kite (0.208), Common Crow (0.160)	–
	Spring	1,424	39	Black Kite (0.209), Common Crow (0.206)	Indian robin, Verditer flycatcher, Variable wheatear

Cultivated Area (Site 3): During summer, 1,800 birds of 23 species were recorded, dominated by common myna (Pi=0.22), black kite (Pi=0.16), and common crow (Pi=0.155). In winter, 1,061 birds of 37 species were observed, with common myna (Pi=0.191) and black kite (Pi=0.171) most common. During spring, 1,304 individuals of 41 species were sighted; common myna (Pi=0.241) and black kite (Pi=0.216) dominated. Least abundant species across seasons included ashy prinia, plain-leaf warbler, and shikra. Botanical Garden (Site 4): During summer, 1,273 birds of 39 species were recorded, with black kite (Pi=0.305) and common myna (Pi=0.178) as the most abundant. In winter, 1,426 birds of 37 species were sighted, with black kite (Pi=0.264) and laughing dove (Pi=0.170) dominant. During spring, 1,255 birds of 38 species were recorded; laughing dove (Pi=0.194) and black kite (Pi=0.171) were the most common. Least abundant species included the Asian koel, the white-browed wagtail, and the spotted owlet (Table 2).

Table 2: Trends in Green Area Bird Populations

Site	Season	Total Individuals	Total Species	Dominant Species (Pi)	Least Observed Species
Teaching Departments (Site 1)	Summer	1,813	34	House Crow (Corvus Splendens) (0.267), Black Kite (Milvus Migrans) (0.161), Common Myna (Acridotheres Tristis) (0.111)	Rufous treepie, Asian koel, Variable wheatear
	Winter	2,060	42	Common Crow (0.183), Common Myna (0.156), Black Kite (0.138)	–

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	Spring	2,002	42	Common Crow (0.188), Black Kite (0.175), Blue Rock Pigeon (Columba Livia) (0.132)	Rufous treepie, Asian koel, Variable wheatear
Residential Area (Site 2)	Summer	1,752	34	Common Crow (0.258), Common Myna (0.210), Black Kite (0.170)	Indian robin, Verditer flycatcher, Variable wheatear
	Winter	1,397	37	Black Kite (0.208), Common Crow (0.160)	–
	Spring	1,424	39	Black Kite (0.209), Common Crow (0.206)	Indian robin, Verditer flycatcher, Variable wheatear
Cultivated Area (Site 3)	Summer	1,800	23	Common Myna (0.220), Black Kite (0.160), Common Crow (0.155)	Ashy prinia, Plain-leaf warbler, Shikra
	Winter	1,061	37	Common Myna (0.191), Black Kite (0.171)	–
	Spring	1,304	41	Common Myna (0.241), Black Kite (0.216)	Ashy prinia, Plain-leaf warbler, Shikra
Botanical Garden (Site 4)	Summer	1,273	39	Black Kite (0.305), Common Myna (0.178)	Asian koel, White-browed wagtail, Spotted owlet
	Winter	1,426	37	Black Kite (0.264), Laughing Dove (0.170)	–
	Spring	1,255	38	Laughing Dove (0.194), Black Kite (0.171)	Asian koel, White-browed wagtail, Spotted owlet

Comparative Trends and Long-Term Changes were done. Overall, black kite, common myna, and house crow remained dominant across all habitats and seasons. However, long-term comparison (1997–2023) shows a stable trend for black kite, declining trends for common crow and common myna, and increasing trends for species such as plain-leaf warbler, yellow-footed green pigeon, and cattle egret (Table 3).

Table 3: Total Seasonal Diversity of the Birds at the University of the Punjab, Quaid-e-Azam Campus, Lahore

Order	Family	Representative Species	Summer (Mean count)	Winter (Mean count)	Spring (Mean count)	Trend 1997–2023
Accipitriformes	Accipitridae	*Milvus migrans* (Black kite)	1265	1134	1146	Stable
Columbiformes	Columbidae	*Spilopelia senegalensis* (Laughing dove)	503	540	540	Stable
Passeriformes	Corvidae	Corvus splendens (Common crow)	1198	594	884	Declining
Passeriformes	Sturnidae	*Acridotheres tristis* (Common myna)	1194	986	888	Declining
Passeriformes	Phylloscopidae	*Phylloscopus neglectus* (Plain leaf warbler)	–	9	9	Increasing
Passeriformes	Pycnonotidae	*Pycnonotus cafer* (Red-vented bulbul)	353	205	198	Stable
Columbiformes	Treronidae	*Treron phoenicopterus* (Yellow-footed green pigeon)	54	89	107	Increasing
Psittaciformes	Psittaculidae	*Psittacula krameri* (Rose-ringed parakeet)	190	99	98	Stable
Pelecaniformes	Ardeidae	*Bubulcus ibis* (Cattle egret)	44	108	104	Increasing
Passeriformes	Nectariniidae	*Cinnyris asiatica* (Purple sunbird)	81	29	38	Stable

The maps indicate a marked increase in built-up areas, particularly after 2012, leading to habitat loss and disturbance. Green patches such as lawns, roadside vegetation, and botanical gardens, though maintained, fail to fully compensate for natural habitat loss. Urban expansion and increased anthropogenic activity were found to negatively affect avian diversity. Regression analysis (to be applied) can quantify the relationship between urbanization intensity and species richness decline. Major changes that occurred after the year 2000 have been marked (Source: Google Earth). The formation of the road is clear. Results indicate a single land without habitat fragmentation (Figure 2).

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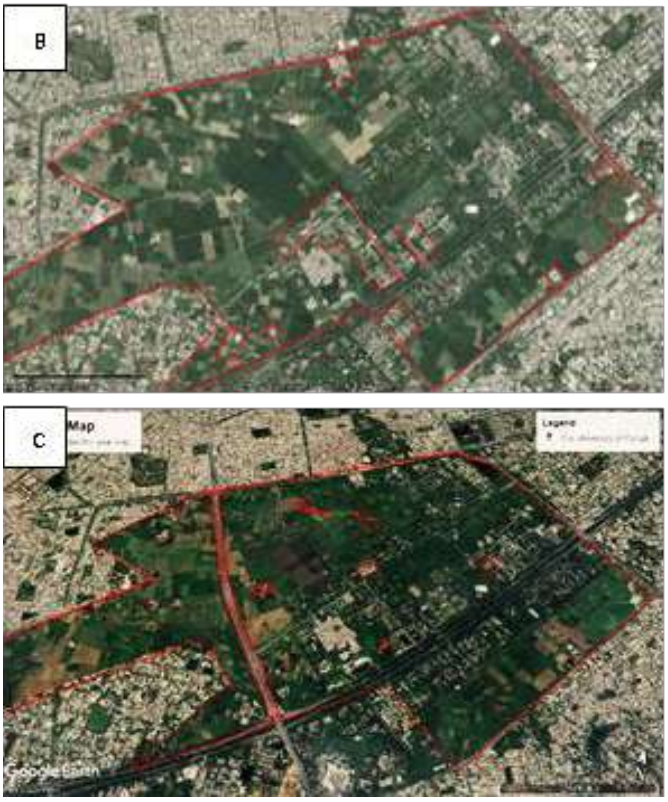

Figure 2: Arial View of the Study Area, University of the Punjab, During the years 2000 (A), 2012 (B), and 2023 (c)

DISCUSSION

Increased urbanization can significantly strain the natural environment, leading to habitat modification, fragmentation, and loss for numerous species. Consequently, biodiversity becomes restricted to limited green spaces within urban areas. The Quaid-e-Azam Campus of the University of the Punjab now serves as a constrained refuge for various bird species. Compared with the earlier study, substantial ecological and structural changes have occurred over time [16]. The total number of recorded bird species has declined from 76 to 65, primarily due to land-use changes. The agricultural area decreased from 426.25 ha to 264 ha, while the urban built-up area expanded from 182.5 ha in 2013 to 387 ha. This decline can be attributed to urban expansion, altered migratory patterns, reduced food availability, and loss of nesting sites affecting specialist species [17]. According to Tanveer *et al.* 64 species of birds were recorded, of which 42 were residents, 6 wintering, and 12 summer breeders that had 2 occasional all-year-round birds [13]. Out of the latter, 33 were passerines and 31 were non-passerines. This was followed by 76 species, 49 of which were residents, 17 winter visitors, 7 summer breeders, 2 passage migrants and 1 vagrant as recorded by Sidra *et al.* [16]. Table 2 in Appendix gives the comparative occurrence and composition of the species. Shannon-Wiener diversity index (H = 2.548) showed that the measure of diversity was moderate with the census density of 2,795 birds/km^2. The rise of the species was not necessarily an increase in the diversity but could have been the result of wastewater sites being added to the survey by Sidra *et al.* [16]. Species movement was also found, with some of the birds not being found in Sidra *et al.* [16], again found in the 2023 survey, indicating temporary displacement and recolonization processes. Table 1 reveals the comparative changes in species. Increase in the scavenger birds especially house crows and black kites may be the result of poor waste disposal habits as residential places attract foraging birds and exterior waste collection sites. The Master Plan of the university shows that the agrarian lands are under gradual transformation in academic and residential structures to allow institutional growth. Such a shift can cause additional species displacement and homogenization of communities [18, 19]. Generalist species like blue rock pigeon, house sparrow, yellow-footed green pigeon, babblers (*Turdoides* spp.), red-vented bulbul, common myna and black kite are common in urban ecosystems and generally adapt to human alterations [20]. With the increase in the number of these adaptive species, they can outcompete or displace specialists that inhabit the habitat, leading to a net decrease in the diversity of avian species on the campus ecosystem. Moreover, waste management information (e.g., volume of garbage, the presence of open waste areas, food waste in the vicinity of hostels and cafeterias, etc.) must be added to compare it with the abundance of scavenger birds (in particular, Corvus splendens and Milvus migrans).

CONCLUSIONS

In conclusion, many species of birds can be found in the Quaid-e-Azam Campus of Punjab University due to its varying habitats. Nonetheless, the growing construction and development demand jeopardizes this biodiversity to the extent of destroying it drastically. The shift of the ecosystems may facilitate the growth of generalist species, as in the case of the omnivorous garbage feeding birds. Such flexible species might also prosper in the case of the suggested modification in land use. Usually, the urban areas are homogeneous in terms of fauna. Changing agricultural areas into buildings can significantly reduce the existing bird species repertoire, even with existing lawns, plantations on the roads, and residential areas.

Authors Contribution

Conceptualization: BNK, MAM
Methodology: BNK, MAM, MNF
Formal analysis: AK, MN, HA
Writing review and editing: BNK, MAM, AK, MN, AT

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Conflicts of Interest

All the authors declare no conflict of interest.

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