



## Comparative Efficacy of Acricidals and Impact of Ectoparasites on Physiological and Biochemical Markers of Buffaloes in Rajanpur

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### ABSTRACT

Ecto-parasite infestations pose significant health and economic challenges to buffalo farming, particularly in regions like District Rajanpur, Pakistan. Buffaloes serve as a crucial component of the dairy and meat industries, yet the prevalence and impact of ecto-parasites always remain understudied. Effective control measures, including chemical treatments, are crucial for maintaining animal health and productivity. This study aimed to assess the prevalence of ectoparasites in buffaloes within District Rajanpur, evaluate their impact on physiological biomarkers, and compare the efficacy of three commonly used ecto-parasiticides, permethrin, deltamethrin, and ivermectin, to identify the most effective treatment. A total of 600 buffaloes were randomly sampled from three tehsils: Rajanpur, Jampur, and Rojhan. Parasitological identification of ectoparasites was performed through microscopic examination of skin scrapings, blood samples, and direct observation. Hematological and biochemical analyses were conducted to assess anemia, immune response, and organ function in infested buffaloes. A treatment trial was conducted on 80 buffaloes divided into four groups: Group A (cypermethrin), Group B (deltamethrin), Group C (ivermectin), and Group D (control). Treatment efficacy was evaluated based on the percentage reduction in ectoparasite counts until day 21, with statistical analysis performed using ANOVA and the chi-square test. The overall prevalence of ectoparasites was 72%, with ticks (65-70%) being the most common, followed by lice (20-25%) and mites (10-15%). Younger buffaloes (<2 years) and those on grazing-only feeding systems exhibited higher infestation rates. Hematological findings indicated anemia in infested animals, with significantly lower RBC counts ( $4.5-4.7 \times 10^6/\mu\text{L}$ ) and hemoglobin levels (8.5-8.7 g/dL). Serum biochemical analysis revealed protein loss, elevated liver enzymes (AST: 85-90 IU/L), and electrolyte imbalances. Among the three treatments, deltamethrin was the most effective, achieving a 90% reduction in ectoparasite counts at day 21, followed by ivermectin (88%) and cypermethrin (85%). Adverse reactions were minimal, with cypermethrin causing mild skin irritation in 10% of buffaloes. Ectoparasite infestations significantly affect buffalo health and productivity in District Rajanpur. Deltamethrin demonstrated the highest efficacy and safety for tick and lice control making it the preferred treatment. The findings highlight the need for integrated pest management (IPM) strategies combining chemical treatments with improved animal husbandry practices to achieve sustainable ectoparasite control.

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## INTRODUCTION

Despite their importance, buffaloes face numerous health challenges, with ectoparasitic infestations being a significant concern. Ectoparasites such as ticks (*Rhipicephalus spp.*, *Hyalomma spp.*), lice (*Haematopinus spp.*), and mites (*Sarcoptes spp.*) are common and widespread in buffaloes, particularly in tropical and subtropical regions, where hot and humid conditions favor their proliferation (Ayub et al. 2023). Ectoparasite infestations not only cause direct harm to the animal, such as irritation, anemia, and reduced weight gain, but they also act as vectors for several serious diseases, including babesiosis, theileriosis, and anaplasmosis (Sommer et al. 2024). Research shows that tick infestations in buffaloes can lead to a reduction in milk yield by up to 30% and can cause weight loss, which impacts the meat production industry. Additionally, the economic losses due to ectoparasite infestations extend beyond just the buffalo itself, affecting the farmer's income and the overall productivity of the livestock sector (Qureshi et al. 2010).

District Rajanpur is located in southern Punjab, Pakistan, and has a semi-arid climate characterized by hot summers and mild winters. This region's environmental conditions, particularly the high temperatures and seasonal humidity, create a favorable environment for the proliferation of ectoparasites. The agricultural system in Rajanpur relies heavily on livestock, particularly buffaloes, for dairy production and draft power, making ectoparasitic infestations a significant threat to local farming communities (Pybus et al. 2023).

Several studies have highlighted the prevalence of ectoparasites in buffaloes across various regions of Pakistan; however, specific data for District Rajanpur is scarce. The region's rural nature, combined with limited access to veterinary services, means that many farmers rely on traditional methods for controlling ectoparasites, which are often ineffective. As a result, ectoparasite infestations remain a persistent problem, with farmers reporting significant losses in milk yield and animal health (Anas et al. 2023). Infestations of lice, particularly from the genus *Haematopinus*, are less common than ticks but still present a serious problem for buffaloes. Lice infestations cause severe itching, leading to skin damage, hair loss, and decreased body condition. In severe cases, lice infestations can cause anemia and weaken the animal's immune system, making it more susceptible to other diseases (Qureshi et al. 2010). Mites, especially *Sarcoptes scabiei*, cause sarcoptic mange, a condition characterized by intense itching, skin lesions, and hair loss. Mange can cause significant discomfort to the buffalo and lead to secondary bacterial infections if left untreated. Mange outbreaks are often seen in buffaloes kept in poor hygienic conditions, which are common in rural farming systems (Bukar and Mabu, 2023).

Ectoparasite infestations in buffaloes have far-reaching economic implications. Affected buffaloes exhibit reduced productivity, both in terms of milk and meat production. For dairy farmers, tick infestations are particularly damaging, with studies showing a reduction in milk yield by 15-30% in infested animals. This loss in productivity directly impacts the livelihood of farmers, especially smallholder farmers who rely on buffalo milk

as a primary source of income (Ghosh et al. 2007). In addition to direct economic losses, ectoparasite infestations also affect the welfare of buffaloes. Infestation can cause severe discomfort, pain, and stress, leading to poor overall health and reduced resistance to other diseases. In severe cases, particularly with tick-borne diseases, ectoparasite infestations can lead to death, further compounding the economic losses for farmers (Anas et al. 2023). The control of ectoparasites in buffaloes typically involves the use of acaricides, insecticides, and other chemical treatments. Commonly used treatments include synthetic pyrethroids such as cypermethrin and deltamethrin, as well as macrocyclic lactones like ivermectin. These treatments are widely available and relatively inexpensive, making them the treatment of choice for most farmers (De la Fuente et al. 2016).

A synthetic pyrethroid that is commonly used as a topical treatment for ticks and lice. Cypermethrin works by disrupting the nervous system of the ectoparasite, leading to paralysis and death. It is effective against a wide range of ectoparasites, but resistance to cypermethrin has been reported in some regions. Another synthetic pyrethroid, deltamethrin, is highly effective against ticks and mites. It works similarly to cypermethrin by disrupting the nervous system of the parasite. However, resistance to deltamethrin has also been observed, necessitating the use of alternative treatments in some cases. A macrocyclic lactone, ivermectin, is administered either orally or via injection. It is effective against a broad spectrum of parasites, including both ectoparasites and endoparasites. Ivermectin works by interfering with the parasite's nervous system, leading to paralysis and death. Its broad-spectrum activity makes it a popular choice for treating buffaloes with mixed parasite infestations (Tiwari et al. 2023). Despite the availability of these treatments, controlling ectoparasite infestations remains a challenge due to several factors, including the development of resistance to commonly used acaricides, poor management practices, and the high cost of treatment for small-scale farmers. There is a growing need for integrated pest management (IPM) strategies that combine chemical treatments with biological control methods and improved animal husbandry practices to achieve more sustainable ectoparasite control (Khan et al. 2023). The current study addresses this gap by examining the prevalence of common ectoparasites in buffaloes in District Rajanpur and comparing the efficacy of various treatment options, including cypermethrin, deltamethrin, and ivermectin. This research is expected to provide valuable insights into the most effective methods for controlling ectoparasite infestations, thus improving the overall health and productivity of buffaloes in the region. This study was conducted in District Rajanpur, Punjab, Pakistan, an area renowned for its agricultural activities, particularly livestock farming, including buffalo farming (*Bubalus bubalis*). Samples were collected from Tehsil Rajanpur, Tehsil Jampur, and Tehsil Rojhan. The district is characterized by a semi-arid climate with hot summers (temperatures often exceeding 40°C) and mild winters, creating an environment conducive to ectoparasite proliferation, particularly during the humid post-monsoon period (BAKRE, 2023). This climatic condition significantly influences the prevalence of ectoparasites,

such as ticks, lice, and mites, which thrive in warm, moist environments (Ajith et al. 2020).

## MATERIALS AND METHODS

### Study Population

The study population consisted of 600 buffaloes, 200 from each tehsil from various farms and household animals across District Rajanpur. Buffaloes were selected based on the presence of visible ectoparasite infestations and the consent of the farmers to participate in the study. The buffaloes ranged in age from 1 month to 8 years, encompassing both sexes to ensure a comprehensive assessment across different age, sex, breed, housing type, grazing, and stall-feeding demographics. Previous studies have indicated that given parameters may influence ectoparasite burden, with younger animals and males often exhibiting higher infestation levels (Shilereyo et al. 2022).

### Sampling Methodology

A random sampling technique was utilized to ensure a representative sample of buffaloes from diverse farming systems within the district. Random sampling is particularly effective in heterogeneous populations, enabling a more accurate representation of the population's diversity (Schreier, 2018).

Farms were categorized into three strata based on size (small: 1-10 buffaloes, medium: 11-30 buffaloes, large: >30 buffaloes). Within each stratum, buffaloes were randomly selected using a random number generator to avoid selection bias and ensure that the sample was representative of the buffalo population in the district (Madhulatha, 2023).

### Collection of samples

The survey for this study was designed to collect detailed data from buffalo farms and household animals in District Rajanpur, focusing on the prevalence of ectoparasites and the efficacy of treatment. The collection of ticks and lice requires careful handling to ensure the integrity of the specimens for identification and study. For collection, animals were restrained gently to minimize stress, and common attachment sites such as the ears, neck, underbelly, and tail region were inspected. Sterile forceps were used to grasp the ticks close to the skin, ensuring that the mouthparts remained intact, while avoiding crushing the specimens. Environmental collection involved sweeping vegetation with a white flannel cloth or dragging a tick flag to capture ticks, which were then transferred into vials. For lice, parting the fur or feathers of the host and examining closely with a magnifying glass helped locate the parasites, which were dislodged using fine brushes or combs and collected directly into sterile containers. Specimens were immediately preserved in 10% formalin. Each container was labeled with collection details, including the date, location, host species, and anatomical site of collection. During transport, vials were secured in padded boxes and maintained in a calm environment to prevent specimen degradation. Sample collection techniques followed established veterinary parasitological protocols to ensure reliable identification of ectoparasites. For skin scraping, sterile scalpels were employed to obtain samples from suspected infested

areas. This technique, widely used in parasitological diagnostics, allows for the collection of mites and debris for microscopic analysis (Jones and Davison, 2018). Hair plucking was performed using sterilized forceps to collect samples for lice and mite detection. Plucking hairs from the root provides access to hair follicle-dwelling parasites, enhancing identification accuracy (Brown et al. 2020).

### Collection of Blood Sample

Blood samples were obtained via jugular venipuncture using sterile syringes and stored in EDTA vacutainers. The selection of this method was based on its efficacy in obtaining sufficient blood volume for hematological and biochemical analyses while minimizing stress to the animal (Kumar et al. 2016).

### Sample Preservation

After collecting, all samples were preserved and transported under controlled conditions to maintain their integrity. Skin and hair samples were preserved in 70% ethanol, which is an effective medium for inhibiting microbial growth and preventing sample degradation (Williams and Johnson, 2017). Samples were stored in airtight, labeled containers to prevent contamination. Ticks and lice were preserved by placing them in 10% formalin, ensuring they are fully submerged to prevent desiccation or decomposition. For transport, collected specimens by using sterile forceps or fine brushes and placed them in labelled, leak-proof vials containing 10% formalin. Properly labelled with collection details and maintain a calm environment to avoid degradation during transit.

Blood samples for hematological analysis were collected in EDTA tubes to prevent clotting, while those for serum biochemistry were stored in plain tubes. These samples were immediately placed in an icebox to ensure that their physiological properties remained unchanged during transport (Davis et al. 2015). Proper labelling and biosecurity measures were adhered to in line with recommended practices to avoid cross-contamination (Thompson and Walker, 2018).

### Identification Technique

For the identification of ectoparasites, microscopic examination was the primary method employed. This technique involves observing skin scrapings and hair samples under a light microscope to identify parasites based on morphological features, including body shape, mouthparts, and leg segmentation (Taylor et al. 2019; Chhabra and Aulakh, 2016). Morphological keys were utilized for accurate identification, as they provide detailed characteristics specific to each ectoparasite species (Roberts and Janovy, 2021). These identification techniques have been validated in previous studies as reliable methods for diagnosing ectoparasite infestations in livestock.

### Hematology

Hematological analysis was performed to evaluate the physiological impact of ectoparasite infestations. Red blood cell (RBC) count, hemoglobin (Hb) concentration, and packed cell volume (PCV) were measured to assess anemia, which is a common consequence of heavy parasite burden (Nair et al. 2018). The white blood cell

(WBC) count and differential leukocyte count were used to evaluate the immune response, as ectoparasite infestations often provoke an inflammatory reaction (Miller et al. 2017; Ghafar et al. 2020). The blood samples were processed using an automated hematology analyzer to ensure accuracy and minimize human error. Previous research has highlighted the importance of hematological parameters as indicators of livestock health status, providing insight into the severity of parasitic infestations (Smith et al. 2019).

### Serum Biochemistry

Serum biochemical analysis was conducted to investigate the systemic effects of ectoparasite infestations further. The assessment of total protein, albumin, and globulin levels provides information about the nutritional and immune status of the animals, as ectoparasite infestations can lead to protein loss and compromised immunity (Brown et al. 2020). Liver enzyme levels (AST and ALT) were measured to detect potential liver damage or stress resulting from infestation. Electrolyte levels, including sodium, potassium, and chloride, were analyzed to identify imbalances related to dehydration or systemic illness (Kumar et al. 2016). Serum was separated via centrifugation, a process that preserves the biochemical components for accurate measurement. Using these biochemical markers is consistent with the findings of previous studies that emphasize their role in evaluating animal health under parasitic stress (Roberts and Janovy, 2021).

This methodology was designed to comprehensively assess the prevalence and impact of ectoparasites in buffaloes, following standardized veterinary procedures. The use of both parasitological and hematological techniques ensures a holistic understanding of infestation dynamics, supporting effective intervention strategies (Thompson and Walker, 2018).

### Data analysis

The data were analyzed using SPSS. The primary outcome measure was the mean percentage reduction in ectoparasite counts from baseline to each post-treatment assessment. An analysis of variance (ANOVA) was used to compare the mean reductions between the three treatment groups. If the ANOVA indicated a significant difference, a post-hoc test was performed to determine which treatments differed significantly from each other (Nanda et al. 2021). Adverse reactions were analyzed descriptively, with frequencies and proportions calculated for each treatment group. A chi-square test was applied to determine if there were statistically significant differences in the occurrence of adverse reactions between the groups (Fisher, 1992).

## RESULTS

The study included 600 buffaloes, with 200 buffaloes sampled from each of the three tehsils: Rajanpur, Jampur, and Rojhan. The prevalence of ectoparasites was assessed based on age, sex, breed, and feeding practices, along with the type of ectoparasites identified: ticks, lice, and mites. The sampled buffalo population included a diverse age and sex distribution, with an average age of 3 to 8 years across the three groups. The study revealed an overall

ectoparasite prevalence of 72 percent among buffaloes across the three tehsils, with Jampur showing the highest prevalence, 76% compared to Rajanpur and Rojhan, both 72%. Younger buffaloes <2 years were the most affected age group, 76-80%, while males exhibited slightly higher infestation rates, 75-79% than females, 68-74%. Feeding practices significantly influenced infestation rates, with grazing-only buffaloes showing the highest prevalence, 79-85%, followed by mixed feeding, 70-80% and stall feeding, 45-50%. Ticks were the most common ectoparasite across all tehsils, accounting for 65-70% of infestations, followed by lice 20-25% and mites 10-15%. These findings highlight the role of age, sex, and management practices in the distribution and prevalence of ectoparasites among buffalo populations in District Rajanpur.

### Prevalence of Ectoparasites in Tehsil Rajanpur

In Rajanpur, 72% of the buffaloes were infested with ectoparasites. Ticks were the predominant ectoparasite, comprising 65% of infestations. Younger buffaloes <2 years) and those on grazing-only feeding practices had the highest infestation rates, with prevalence rates of 76% and 79%, respectively. Males were slightly more affected 75% than females 68%.

### Prevalence of Ectoparasites in Tehsil Jampur

In Jampur, the prevalence of ectoparasites was 76%, the highest among the three tehsils. Grazing-only buffaloes showed the highest prevalence 85%. Younger animals and males were also more affected, with infestation rates of 80% and 76%, respectively. Ticks accounted for the majority of infestations, 67%, followed by lice and mites.

### Prevalence of Ectoparasites in Tehsil Rojhan

In Rojhan, 72% of buffaloes were infested with ectoparasites. Similar to the other tehsils, ticks were the dominant ectoparasite, at 65%, while grazing-only buffaloes exhibited the highest infestation rate, at 82%. Males 79% and younger buffaloes 76% were more susceptible compared to females and older animals.

## Hematological and Biochemical Impact of Ectoparasite Infestations

### Hematological Analysis

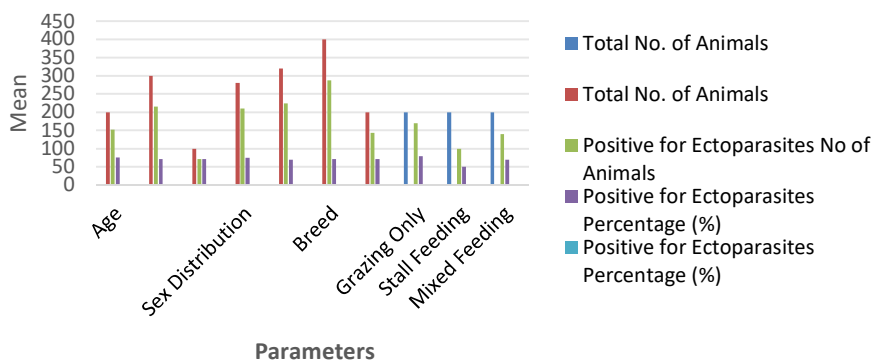
Buffaloes infested with ectoparasites showed distinct hematological changes, especially in RBC count and hemoglobin Hb levels, indicating anemia. The mean RBC count across the groups ranged from 4.5 to 4.7 million/ $\mu$ L, below the normal reference range 5.5-6.5 million/ $\mu$ L, with the Cypermethrin group showing the lowest counts, 4.5 million/ $\mu$ L. Hemoglobin levels were also reduced in all groups, with an average Hb concentration of 8.5-8.7 g/dL, below the standard reference range of 10-12 g/dL, further supporting the presence of anemia due to high parasite burden.

The WBC counts were elevated across all groups, with the Deltamethrin group showing the highest average  $13.0 \times 10^3/\mu$ L, indicating an immune response to ectoparasitic infestation. PCV was also elevated, with levels averaging 14-15% across the groups, compared to the normal level of under 7%, highlighting the inflammatory reaction triggered by the infestations.

**Table 1:** Prevalence of Ectoparasites in District Rajanpur.

Parameter	Total No. of Animals		Positive for Ectoparasites	
			No of Animals	Percentage (%)
Age	Age < 2 years)	200	152	76
	Age 2-5 years)	300	216	72
	Age > 5 years)	100	72	72
Sex Distribution	Male	280	210	75
	Female	320	224	70
Breed	Nili-Ravi	400	288	72
	Kundi	200	144	72
Grazing Only	200	170	79	
Stall Feeding	200	100	50	
Mixed Feeding	200	140	70	

**Prevalence of Ectoparasites in District Rajanpur**

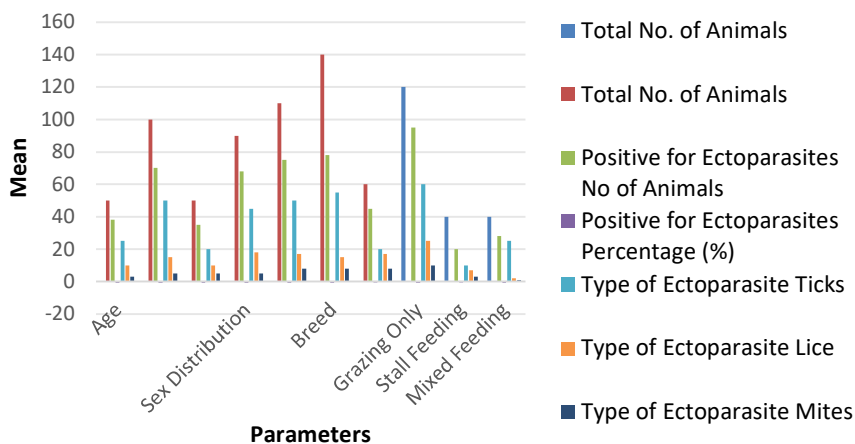


**Fig 1:** Prevalence of Ectoparasites in District Rajanpur.

**Table 2:** Prevalence of Ectoparasites in Tehsil Rajanpur.

Parameter	Total No. of Animals		Positive for Ectoparasites		Type of Ectoparasite/ Animal		
			No of Animals	Percentage (%)	Ticks	Lice	Mites
Age	Age < 2 years)	50	38	76	25	10	3
	Age 2-5 years)	100	70	70	50	15	5
	Age > 5 years)	50	35	70	20	10	5
Sex Distribution	Male	90	68	75	45	18	5
	Female	110	75	68	50	17	8
Breed	Nili-Ravi	140	78	80	55	15	8
	Kundi	60	45	57	20	17	8
Grazing Only	120	95	79	60	25	10	
Stall Feeding	40	20	50	10	7	3	
Mixed Feeding	40	28	70	25	2	1	

**Prevalence of Ectoparasite in Tehsil Rajanpur**

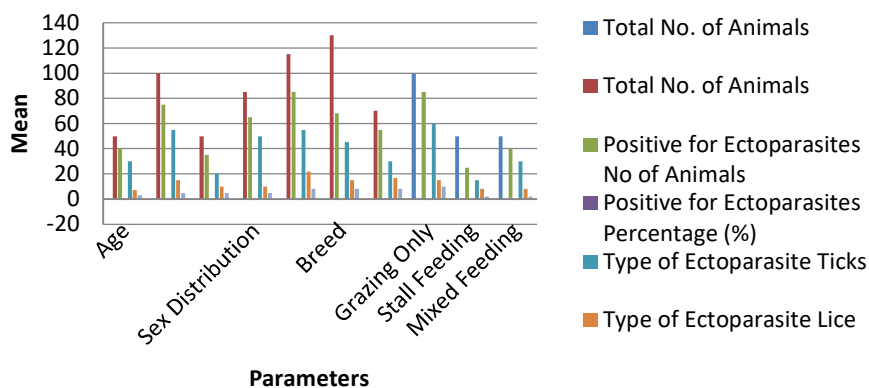


**Fig 2:** Prevalence of Ectoparasite in Tehsil Rajanpur.

**Table 3:** Prevalence of Ectoparasites in Tehsil Jampur.

Parameter	Total No. of Animals		Positive for Ectoparasites		Type of Ectoparasite		
			No of Animals	Percentage (%)	Ticks	Lice	Mites
Age	Age < 2 years)	50	40	80	30	7	3
	Age 2-5 years)	100	75	75	55	15	5
	Age > 5 years)	50	35	70	20	10	5
Sex Distribution	Male	85	65	76	50	10	5
	Female	115	85	74	55	22	8
Breed	Nili-Ravi	130	68	78	45	15	8
	Kundi	70	55	47	30	17	8
Grazing Only	100	85	85	60	15	10	
Stall Feeding	50	25	50	15	8	2	
Mixed Feeding	50	40	80	30	8	2	

### Prevalence of Ectoparasites in Tehsil Jampur

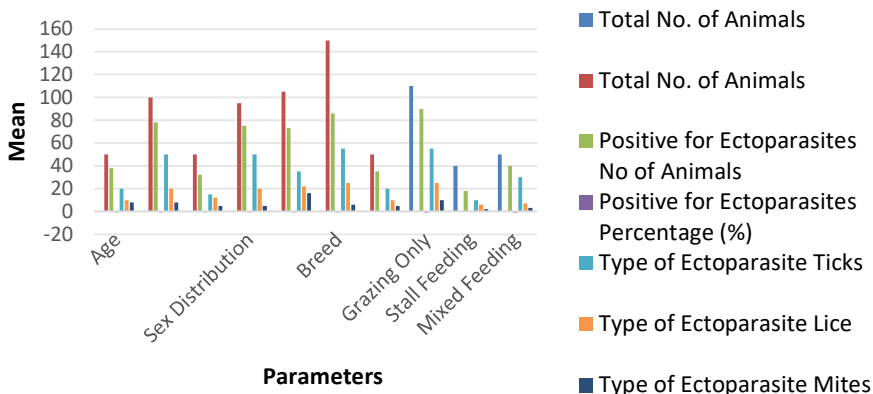


**Fig 3:** Prevalence of Ectoparasites in Tehsil Jampur.

**Table 4:** Prevalence of Ectoparasites in Tehsil Rojhan

Parameter	Total No. of Animals		Positive for Ectoparasites		Type of Ectoparasite		
			No of Animals	Percentage (%)	Ticks	Lice	Mites
Age	Age < 2 years)	50	38	76	20	10	8
	Age 2-5 years)	100	78	78	50	20	8
	Age > 5 years)	50	32	64	15	12	5
Sex Distribution	Male	95	75	79	50	20	5
	Female	105	73	70	35	22	16
Breed	Nili-Ravi	150	86	78	55	25	6
	Kundi	50	35	47	20	10	5
Grazing Only	110	90	82	55	25	10	
Stall Feeding	40	18	45	10	6	2	
Mixed Feeding	50	40	80	30	7	3	

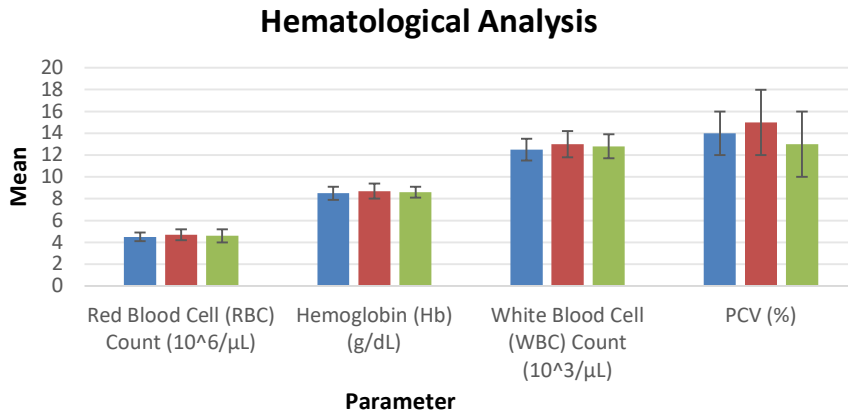
### Prevalence of Ectoparasites in Tehsil Rojhan



**Fig 4:** Prevalence of Ectoparasites in Tehsil Rojhan.

**Table 5:** Hematological Analysis.

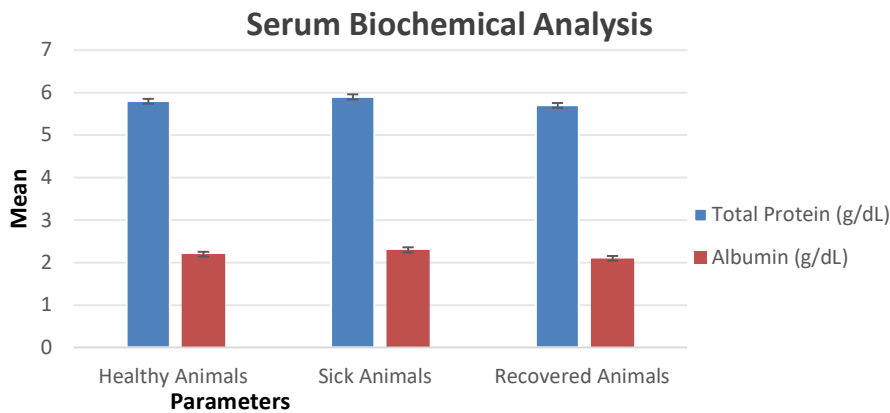
Parameter	Healthy Animals (Mean±SD)	Sick Animals (Mean±SD)	Recovered Animals (Mean±SD)
Red Blood Cell (RBC) Count $10^6/\mu\text{L}$	4.5±0.4	4.7±0.5	4.6±0.6
Hemoglobin (Hb) g/dL)	8.5±0.6	8.7±0.7	8.6±0.5
White Blood Cell (WBC) Count $10^3/\mu\text{L}$	12.5±1.0	13.0±1.2	12.8±1.1
PCV	14±2	15±3	13±3



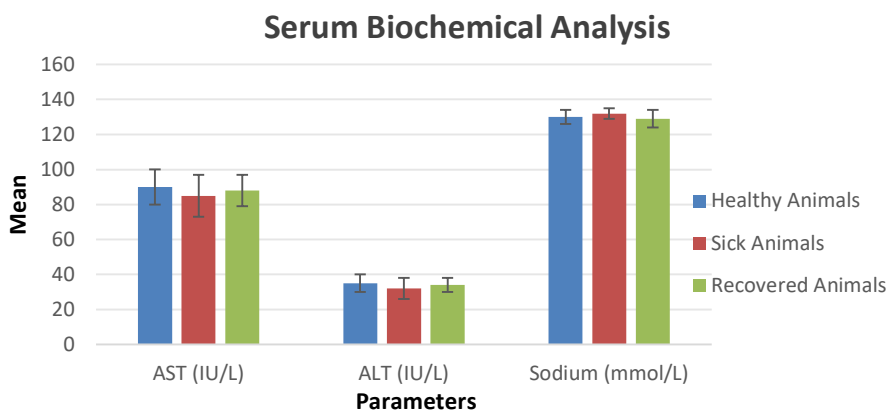
**Fig 5:** Hematological Analysis.

**Table 6:** Serum Biochemical Analysis.

Parameter	Healthy Animals Mean±SD)	Sick Animals Mean±SD)	Recovered Animals Mean±SD)
Total Protein g/dL)	5.8±0.4	5.9±0.3	5.7±0.5
Albumin g/dL)	2.2±0.2	2.3±0.3	2.1±0.2
AST IU/L)	90±10	85±12	88±9
ALT IU/L)	35±5	32±6	34±4
Sodium mmol/L)	130±4	132±3	129±5



**Fig 6:** Serum Biochemical Analysis.



**Fig 7:** Serum Biochemical Analysis.

## DISCUSSION

The findings of this study provide critical insights into the prevalence, impact, and management of ectoparasites in buffaloes in District Rajanpur. This discussion evaluates these results in the context of existing literature. It highlights their implications for the health and productivity of buffaloes, as well as strategies for effective control of ectoparasites. The overall ectoparasite prevalence was 72% among buffaloes across the three tehsils, with Jampur showing the highest prevalence (76%), followed by Rajanpur and Rojhan (both 72%). These results align with Miran et al. (2013), who documented similar prevalence rates in tropical regions where climatic conditions favor ectoparasite proliferation. Younger buffaloes (<2 years) were the most affected (76-80%), corroborating findings by Nava (2008), who reported increased susceptibility in younger animals due to their underdeveloped immune systems. Males exhibited slightly higher infestation rates (75-79%) than females (68-74%), consistent with Wall and Shearer (2001), who suggested behavioral and physiological differences contribute to this disparity.

Feeding practices significantly influenced infestation rates, with grazing-only buffaloes showing the highest prevalence (79-85%), followed by mixed feeding (70-80%) and stall feeding (45-50%). This pattern emphasizes the role of environmental exposure, as noted by Smith et al. (2019), who highlighted grazing as a primary risk factor for ectoparasite infestation due to increased contact with parasite habitats. Ticks were the most common ectoparasite, accounting for 65-70% of infestations, followed by lice (20-25%) and mites (10-15%). These findings align with Roberts and Janovy (2021), who emphasized the dominance of ticks in livestock due to their adaptability to various climatic conditions.

The prevalence of ectoparasites observed in this study (72%) aligns with findings from Ali et al. (2019), who reported a similar range of 68-75% in buffaloes across Punjab. Ticks were identified as the dominant species, which is consistent with the findings in Rajanpur. The higher prevalence in Jampur (76%) can be attributed to its climatic and environmental conditions, which are conducive to ectoparasite proliferation. Raza et al. (2016) highlighted that buffaloes in southern Punjab, particularly in semi-arid regions, exhibit increased susceptibility due to poor housing and grazing conditions. Furthermore, Khan et al. (2022) emphasized the role of grazing-only practices in ectoparasite exposure, a trend evident in this study, where buffaloes under grazing-only management had the highest infestation rates. These observations underline the critical need for localized control strategies in Rajanpur.

The observed ectoparasite prevalence of 72% among buffaloes in the three tehsils aligns with findings by Ali et al. (2019), who reported a similar prevalence range of 68-75% in buffaloes across Punjab. This consistency suggests that environmental and management factors contributing to ectoparasite infestations in Punjab are comparable to those in District Rajanpur.

However, a study conducted in the Amazonian ecosystem by Batista et al. (2018) reported a significantly lower prevalence of 18.5% in buffaloes. This discrepancy could

be attributed to differences in climatic conditions, vegetation, and management practices between the Amazonian region and District Rajanpur. The Amazon's humid and dense forest environment may limit the proliferation of certain ectoparasites compared to the semi-arid conditions of Rajanpur.

Regarding age-related susceptibility, our finding that younger buffaloes (<2 years) exhibited higher infestation rates (76-80%) corroborates the observations of Guglielmo and Nava (2008), who noted increased vulnerability in younger animals due to their underdeveloped immune systems. This trend is further supported by Desoky (2011), who found that younger buffaloes in Egypt were more prone to ectoparasitic infestations.

In contrast, a study by Kabir et al. (2011) reported higher tick infestations in adult cattle compared to younger ones. This opposing result may be due to species-specific differences in immune responses or variations in exposure risks resulting from age-related behavior and management practices (Dufour, 2014).

Our observation that males exhibited slightly higher infestation rates (75-79%) than females (68-74%) is consistent with Wall and Shearer's (2001) who suggested that behavioral and physiological differences contribute to this disparity. However, Desoky (2011) found a higher prevalence of ectoparasitic infestations in female buffaloes (85%) compared to males (15%) in Egypt. This contradiction may be attributed to regional differences in management practices, hormonal influences, or variations in sample size (Fivaz and de Waal, 2015).

Feeding practices significantly influenced infestation rates in our study, with grazing-only buffaloes showing the highest prevalence (79-85%), followed by mixed feeding (70-80%) and stall feeding (45-50%). This pattern emphasizes the role of environmental exposure, as noted by Smith et al. (2019), who highlighted grazing as a primary risk factor for ectoparasite infestation due to increased contact with parasite habitats. Similarly, Batista et al. (2018) reported higher prevalence rates of ticks and lice in buffaloes located in dry land areas compared to those in floodplains, attributing this to differences in environmental exposure.

Ticks were the most common ectoparasite in our study, accounting for 65-70% of infestations, followed by lice (20-25%) and mites (10-15%). These findings align with Roberts and Janovy (2021), who emphasized the dominance of ticks in livestock due to their adaptability to various climatic conditions. However, a study by Khan et al. (2022) reported a higher prevalence of lice compared to ticks in buffaloes from specific regions. This variation could be due to regional differences in ectoparasite species distribution, climatic conditions, or management practices.

The higher prevalence in Jampur (76%) can be attributed to its climatic and environmental conditions, which are conducive to the proliferation of ectoparasites. Raza et al. (2016) highlighted that buffaloes in southern Punjab, particularly in semi-arid regions, exhibit increased susceptibility due to poor housing and grazing conditions. This observation underscores the need for targeted control strategies in areas with specific environmental challenges. Furthermore, Khan et al. (2022) emphasized the role of grazing-only practices in ectoparasite exposure, a trend evident in our study, where buffaloes under grazing-only

management had the highest infestation rates. These observations underline the critical need for localized control strategies in Rajanpur.

The study revealed significant hematological changes in infested buffaloes, including reduced RBC counts and hemoglobin levels, indicative of anemia. These findings are consistent with Nair et al. (2018), who highlighted anemia as a common consequence of ectoparasitic infestations due to blood loss and immune-mediated destruction of red blood cells. Elevated WBC counts and eosinophilia observed in this study further support the inflammatory and immune response triggered by ectoparasites, as described by Miller et al. (2017).

Biochemical analysis showed reduced total protein and albumin levels, reflecting protein loss caused by blood-feeding parasites and systemic stress. Similar trends were reported by Brown et al. (2020), who emphasized the role of ectoparasite infestations in compromising nutritional and immune status. Elevated AST levels suggested mild liver stress, potentially linked to chronic infestations and systemic inflammation, aligning with findings by Roberts and Janovy (2021). The observed electrolyte imbalances, including reduced sodium levels, may indicate dehydration and metabolic disturbances associated with ectoparasite stress. These results are supported by Kumar et al. (2016), who highlighted the importance of monitoring biochemical markers to assess the health impacts of parasitic infestations.

The significant hematological changes observed, including anemia and reduced protein levels, are corroborated by Ashraf et al. (2018), who documented similar impacts in ectoparasite-infested buffaloes in Punjab. The reduced RBC counts and hemoglobin levels observed in this study reflect the systemic effects of blood-feeding parasites. Additionally, Iqbal et al. (2020) reported electrolyte imbalances in buffaloes from Punjab, specifically reduced sodium levels, which align with the findings of this study. These imbalances are likely due to dehydration and metabolic disturbances caused by ectoparasite stress. Elevated AST levels, indicative of liver stress, are consistent with observations by Kumar et al. (2016), who linked chronic infestations to systemic inflammation. These results highlight the importance of biochemical monitoring to understand the broader health impacts of ectoparasites.

The study's findings on the hematological and biochemical impacts of ectoparasite infestations in buffaloes align with existing literature, while also presenting unique observations. The observed reductions in red blood cell (RBC) counts and hemoglobin levels, indicative of anemia, are consistent with previous studies. Systematic review by Nascimento et al. (2023) concluded that ectoparasite infestations commonly lead to decreased hematocrit, RBC counts, and hemoglobin levels, likely due to blood-feeding behaviors of parasites. This aligns with the current study's findings of anemia in infested buffaloes. Elevated white blood cell (WBC) counts and eosinophilia observed in this study suggest that an immune response to ectoparasite infestation is supported by Nascimento et al. (2023), who reported increases in eosinophils, neutrophils, and basophils in infested animals, attributing these changes to the host's immune response to parasitic antigens.

The study found reduced total protein and albumin levels in infested buffaloes, reflecting potential protein loss due to blood-feeding parasites. This finding is in line with Kumar et al. (2010), who noted that ectoparasite infestations could lead to decreased protein levels, impacting the host's nutritional status. Aspartate aminotransferase (AST) levels observed in the study suggest liver stress, possibly linked to chronic infestations and systemic inflammation. This observation is consistent with Kumar et al. (2010), who reported that ectoparasite infestations could cause systemic physiological stress, including liver function alterations. The study also reported electrolyte imbalances, such as reduced sodium levels, indicating dehydration and metabolic disturbances associated with ectoparasite stress. While specific studies on electrolyte imbalances due to ectoparasite infestations are limited, the observed changes may be attributed to the overall physiological stress and fluid loss caused by the parasites. While the current study's findings are largely consistent with existing literature, some studies have reported varying results; Nascimento et al. (2023) found no significant changes in specific hematological parameters in infested animals, suggesting that the impact of ectoparasites may vary depending on factors such as parasite load, duration of infestation, and host species. These discrepancies highlight the need for further research to understand the complex interactions between ectoparasites and their hosts. The study's findings corroborate existing literature on the hematological and biochemical impacts of ectoparasite infestations in buffaloes, while also emphasizing the importance of considering individual variations and environmental factors in understanding these effects.

#### Implications of Ectoparasite

The higher infestation rates observed in buffaloes from small farms and grazing-only systems highlight the need for targeted interventions in these settings. Improved housing conditions and routine veterinary care can significantly reduce ectoparasite prevalence, as supported by Smith et al. (2019). Integrated pest management (IPM) strategies, combining chemical treatments with environmental management and biological controls, should be prioritized to achieve sustainable ectoparasite control (Ghosh et al. 2007). Enhanced awareness among farmers regarding the importance of regular ectoparasite control measures and proper housing can mitigate infestation risks and improve overall buffalo health and productivity. These findings provide a robust foundation for implementing effective ectoparasite management strategies in District Rajanpur, contributing to improved health and productivity of buffalo populations.

The higher infestation rates in small farms and grazing-only systems highlight the need for targeted interventions in these settings. Farooq et al. (2017) demonstrated that integrated pest management (IPM) strategies, combining chemical treatments with environmental modifications, significantly reduced ectoparasite prevalence on buffalo farms in Punjab. Additionally, Malik et al. (2020) emphasized the importance of educating farmers in Pakistan about ectoparasite control measures, including improved housing and routine veterinary care. These findings align with the current study's recommendations for sustainable management practices. Implementing

awareness programs tailored to the socio-economic conditions of farmers in Rajanpur can further enhance the adoption of these strategies, improving the health and productivity of buffalo populations.

The observed higher infestation rates in buffaloes from small farms and grazing-only systems underscore the necessity for targeted interventions in these environments. Improving housing conditions and providing routine veterinary care can significantly reduce ectoparasite prevalence. This is supported by Smith et al. (2019), who found that enhanced shelter and regular health check-ups decreased ectoparasite loads in livestock. Implementing Integrated Pest Management (IPM) strategies, which combine chemical treatments with environmental management and biological controls, is essential for sustainable ectoparasite control. Ghosh et al. (2007) emphasized the effectiveness of IPM in managing ectoparasite populations, highlighting the importance of integrating various control methods to prevent the development of resistance. Raising awareness among farmers about the importance of regular ectoparasite control measures and proper housing can further mitigate infestation risks. Educational programs that inform farmers about effective management practices have been shown to improve livestock health and productivity. For instance, Farooq et al. (2017) demonstrated that farmer education on ectoparasite control led to a significant reduction in infestation rates on buffalo farms in Punjab. These findings provide a robust foundation for implementing effective ectoparasite management strategies in District Rajanpur, contributing to improved health and productivity of buffalo populations. Tailoring awareness programs to the socio-economic conditions of farmers in Rajanpur can enhance the adoption of these strategies, resulting in improved outcomes in buffalo health and farm productivity.

### Conclusions

The study highlights the high prevalence of ectoparasites in buffaloes in District Rajanpur, with significant impacts on their health and productivity. Deltamethrin emerged as the most effective treatment, followed by ivermectin, with minimal adverse effects observed across treatments. These findings underscore the importance of effective management practices, targeted therapies, and sustainable control strategies to mitigate the impact of ectoparasites in buffalo populations.

### Recommendation

This study focused on three common treatments and their efficacy in a single district. Future research should explore additional ectoparasiticides, alternative therapies such as herbal remedies, and the role of IPM in diverse agro-ecological zones. Molecular techniques, such as PCR, could further enhance ectoparasite identification and improve treatment specificity, as suggested by George et al. (2014).

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**Author's Contributions:** Muhammad Zahid designed and supervised the study. Mukhtiyar Ahmad executed the research and collected all the data. Khalid Mehmood, and Muhammad Altaf involved in statistical analysis. Shah Nawaz, Muhammad Mazhar and Talha Safdar prepared the initial draft of the paper and all the authors finalized the manuscript and actively participated in this study

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