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Biodiversity of invertebrates in association with large ruminants in the vicinity areas of Tolipir National Park

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Abstract. The present study was carried out to record the biodiversity of arthropods associated with ruminants from the vicinity area of Tolipir National Park Azad, Kashmir, Pakistan. The survey was conducted from March to October 2018, on arthropods of ruminants and their infestation in dairy form and domestic cows. A total of 500 ruminants (cows and buffalos) were observed from ten study sites: Ghorimar, Noor Kot, Kotari, Darak, Alisojal, Kahoo Kot, Dhahol Dhok, Hussain Kot, Pir Kot, and Panjal. We recorded two lice species (*Pediculus* sp. and *Bovicola bovis*), four tick species (*Hyalomma anatolicum*, *Rhipicephalus microplus*, *Boophilus microplus*, *Haemaphysalis salcata*), one flea species (*Ctenocephalus canis*) and one unidentified lice species. The supportive data of large ruminants such as age, sex, nutrition condition and breed were also recorded. Among five hundred cows, 297 (59.4 %) animals were infected with insects and 40% (203) animals were infected with ticks, lice, and flea. As a results of seasonal variation, insect infestation was higher in the summer season than in the winter. The present study provides baseline data for insects, ticks, lice, and flea infestation for further management of arthropods associated with ruminants.

Keywords: invertebrates, infestation, Tolipir National Park, cows and buffalos, Pakistan.

Introduction

The parasitic arthropods (insects, mites, lice, and fleas) of large ruminants live in burrows, punctures, caves, crevices, and humid places, and breed in moist places (Bram et al. 2002). These arthropods cause spreading of diseases, and as their parasitic characteristic they depend on the host and nourish by sucking blood, causing skin injuries and skin problems in livestock communities. Parasitic arthropods transmit disease-causing agents to animals such as viruses, bacteria, protozoa, or many other micro-organisms. Obligate parasites live in burrows, caves, ditches, or on the skin of the animals (Petney et al. 2007). Livestock represents one of the most important parts of Pakistan`s economy (energy, food, raw materials, fertilizers for crops) and is on high threat due to various arthropods (Birrell et al. 2020). The arthropods of ruminants belong to the phylum Arthropoda and include two classes, class Insecta (e.g., lices - Phthiraptera) and class Arachnida (i.e., ticks, as hard ticks (Argasidae), soft ticks (Ixodidae), scab mites (Psoroptidae)), and they transmit diseases as typhus, fever, allergic reaction, yellow fever. Insects and other arthropods transmit parasites which cause diseases (Birrell et al. 2020). Several studies on arthropods such as biodiversity of insects (Faiz et al. 2018, Bagaturov et al. 2020) or biodiversity of moths (Hassan et al. 2019) were taken in Azad Jammu and Kashmir, but studies on the association of arthropods with animals are lacking. The present study`s main objective was to estimate the diversity of arthropods associated with large ruminants.

Material and Methods

Study area

Tolipir National Park is a reserve of natural or semi-natural land, declared or owned by the national government, set aside for human recreation and enjoyment, but protected from most human caused innovations/exploitation. The selected areas near Tolipir National Park are: Ghorimar, Ali Sojal, Noor Kot, Pir Kot, Hussain Kot, Darak, Kotari, Kahoo Kot, Dhahol Dhok and Dhara (Fig. 1). The study areas are linked with Rawalpindi and Islamabad via Goyain Nala and Tain roads. The average temperature of the study area is 24.0˚ C, and January is the coldest month of the year with the temperature range from 5˚ C to 0˚ C in the snow season, climate is temperate sub-humid, with annual rainfall ranging from 500-2,000 mm (AWC 2021).

Collection of samples

The indigenous and cross breed animals were closely visited, and their skin was examined by parting the hairs against the normal direction for observing the insects or other parasites. Samples were collected from different parts of the animals, by hand picking method with forceps (detachment of ticks), and by chemicals. The collected samples were preserved in 70% alcohol in clean and labeled glass vials. The collected samples were identified subsequently (Soulsby 1982).

Statistical analysis.

The statistical analysis was performed to find significant differences between the infected and the non-infected population (area wise prevalence, gender wise prevalence, host wise prevalence, age wise prevalence, treatment wise prevalence, seasonal prevalence, insect infestation of animal hygiene management, nature of livestock and insect associations, feeding mode and insect infestation, nutrition base prevalence of insect infestation, prevalence of insects in ruminants) by using Xlstat (vers. 4.02).

Results

A total of 1800 invertebrates were collected from 500 ruminants (cows and buffalos) from ten study sites: Ghorimar, Noor Kot, Kotari, Darak, Alisojal, Kahoo Kot, Dhahol Dhok, Hussain Kot, Pir Kot, and Panjal; the invertebrates were identified by using the keys of Soulby (1982) (Table 1). We recorded two lice species (*Pediculus* sp. and *Bovicola bovis*), four tick species (*Hyalomma anatolicum*, *Rhipicephalus microplus*, *Boophilus microplus*, *Haemaphysalis salcata*), one flea species (*Ctenocephalus canis*) and one unidentified lice species.

Figure 1. Biotope of study area.

Table 1. Characteristics studies for identification of ticks (based on Soulby, 1982).

	Characteristics Observed	Identification/ Taxa
1	Cornua are distinct, central i. plates are indistinct, three ii. accessory adanal plates	*Rhipicephalus* spp.
2	Palp article 2, dorsal spur absent, lateral extensions are small	*Haemaphysalis* spp.
3	Margin of body with i. definite sutural line. ii. capitulum inferior, iii. scutum absent, margin of body thick iv. rounded without sutural line. v. mouth parts are small	*Boophilus* spp.
4	Legs are pale white in colours, i. eyes and palp are present. ii. festoons are pale white color iii. mouth parts are forwarded	*Hyalomma* spp.

Area wise prevalence

The total observed animals in Ghorimar were 60, of which 40 (66%) were infested, and 20 (33%) were non- infested, while at the study site Noor Kot 50 animals were observed, of which 40 (80%) animals were infested with insects. At Kotari study site we examined 100 animals of which 20 (20%) animals were infested. The total observed animals in Darak were 10, of which 5 (50%) were infested with ticks, lice, and mites. In Kahoo Kot 50 animals were examined, of which 30 (60%) were infested with ticks, mites, lice, and fleas. In Dhahol Dhok 70 animals were examined, and 50 (83%) were infested. The total observed animals in Hussainkot were 70, and 50 (83%) animals were infested with ticks, mites, lice, and fleas.

The total observed animals in Pir Kot were 10, of which 8 (80%) were infested with ticks, lice, and mites. In Panjal 70 animals were examined, and 50 (71%) were infested by ticks, lice, mites, and fleas (Table 2).

Table 2. Area-wise prevalence insects with large ruminants

Areas	Infected animals	Non-Infected animals	Total	GPS coordinates
Ghorimar	40(60%)	20 (20%)	60	N33° 56′ 42.01", E73° 57' 26.07"
Noor Kot	40 (80%)	10 (20%)	50	N33° 53' 11.25" E73° 51' 38.82"
Kotera	20 (20%)	80 (80%)	100	N33° 46' 51.39", E73° 46' 50.47"
Darak	5 (50%)	5 (50%)	10	N33°49' 26.38", E73° 47' 08.79"
Alisojal	4 (40%)	6 (60%)	10	N33° 52' 12.20", E73° 52' 43.62"
Kahoo Kot	30 (60%)	20 (40%)	50	N33° 53' 46.14", E73°"52'"21.68"
Dhahol dhok	50 (83%)	20 (16%)	70	N33° 52' 33.74", E73°" 51' 55.80"
Hussain kot	50 (83%)	20 (16%)	70	N33° 50' 41.76", E73° 46' 56.52"
Pir Kot	8 (80%)	2 (20%)	10	N 33° 53' 34.32", E73° 51' 52.66"
Panjal	50 (71%)	20 (28%)	70	N33° 53' 34.32", E73° 51' 52.66"

Gender-wise prevalence

A total of 60 male ruminants were observed out of which 15 (25%) male animals were infested and 45 (75%) animals were non-infested. The observed female animals were 440, of which 330 (75%) were infested, while 110 (25%) were non-infested. The highly significant differences (<0.05) among genders of the studied animals are shown in Table 3. Females were more affected due to pregnancy.

Table 3. Gender-wise prevalence of insects with large ruminants

Gender	Males	Females	P(T<=t) two-tail
Infested Animals	15 (25%)	330 (75%)	0.37
Non- Infested Animals	45 (75%)	110 (25%)	
Total	60	440	

Age-wise prevalence

Animal belonging to different age groups were observed. In the first group, which included 60 animals between 6 months to 1 year old, infested animals were 8 (13%) while non-infested were 52 (86%).

The second group were 2 to 3 years old animals; in this group the total observed animals were 160, out of which 100 (62%) were infested and 60 (37.5%) were non-infested.

In the third group (animals older than 4 years old) the total observed animals were 280, out of which 180 (64%) were infested and 100 (35.7%) were non-infested.

Significant ($p<0.05$) differences represented between different age groups of infested and non-infested animals are shown in Table 4.

Table 4 Age-wise prevalence of insect association with large ruminants

Age Group	1 Year	2-3 Years	P(T<=t)	2-3 Years	>4 year	P(T<=t)
Infested animals	8 (13%)	100 (62%)	0.38	100 (62%)	180 (64%)	0.2
Non-infested animals	52(86%)	60 (37.5%)		60 (37.5%)	100 (35.7%)	
Total	60	160		160	280	

Host-wise prevalence

Two types of hosts (buffaloes and cows) were observed. The total observed buffaloes were 300, of which the infested buffaloes were found 220 (73%) and non-infested were 80 (26%). We also examined 200 cows, out of which infested cows were 190 (95%) while non-infested were 10 (5%). Significant ($p=0.05$) differences between the studied animal groups are shown in Table 4. Cows are less infested than buffalos, due to presence of dense hairs. Among bovines, cows (95%) are more infested than buffaloes (73%). We studied Sahiwali, Cholistani, Holstein, Friesian cows, and their breed. There were significant differences ($p\leq0.05$) between the two hosts (cows and buffalos) (Table 5).

Table 5. Host-wise prevalence of insects in large ruminants

Host	Cows	Buffaloes	P(T<=t)
Infested animals	190 (95%)	220 (73%)	0.24
Non-infested animals	10 (5%)	80 (26%)	
Total	200	300	

Treatment-wise prevalence

In this study we found the accurate percentage of animals' treatment effected for arthropods, according to schedule wise treatment, 1 year treatment schedule of 100 animals. Out of 100 animals treated 70 (70%) animals were infested and 30 (30%) animals were non- infested. In the 6-month treatment the total observed animals were 150 specimens, out of which infested animals were 100 (66.6%) while non-infested animals were 50 (33.3%). In the three-month treatment total observed animals were 250 out of which 160 (64%) animals were infested and 90 (36%) were non-infested. The significance of differences ($p<0.05$) between animals with different treatment schedule are shown in Table 6.

Seasonal prevalence of arthropods

The total observed animals in the winter were 140, out of which 90 (64.2%) were infested and 50 (35.5%) animals were non-infested. The total observed animals in the summer were 150 out of which 100 (67%) animals were infested and 50 (33%) animals were non-infested. The total observed animals in monsoon were 210, out of which 130 (61%) animals were infested and 80 (38%) animals were non-infested. The significance ($p<0.05$) of seasonal research in studied animals is shown in Table 7. There are significant differences between the seasons.

Animal hygiene and management

The animals were observed in different management conditions. Animals observed in poor hygiene conditions were 90, out of which 70 (77%) were infested and 20 (22%) were non-infested. In normal management we observed a total of 156 animals, of which only 6 (3%) animals were infested, and 150 (96) animals were non-infested. In good management we observed 254 animals, of which 54 (5%) were infested while 200 (78.7%) animals were non-infested. The significance of animals' hygiene management in studied animals is presented in Table 8.

Nature of livestock and insect association

A total of 230 buffaloes flock was observed, out of which 180 (78%) animals were infested while 50 (21%) animals were non- infested. In the cow flock observed were 80 animals, out of which 50 (62%) animals were infested while 30 (37%) animals were non- infested. The mixed flock livestock had 190 animals, out of which 140 (73%) animals were infested while 50 (26%) animals were non- infested. The level of significance between infested cows and buffalos in studied animals is presented in Table 9.

Feeding mode and insect infestation

We observed 105 animals in the grazing stall, out of which 15 (14%) animals were infested while 90 (85%) animals were non-infested. Out of the 120 pasture grazing animals, 70 (58%) were infested and 50 (42%) were non-infested. The

observed animals in mixed grazing were 275, out of which 200 (53%) animals were infested and 75 (33%) were non-infested. The food wise prevalence shows significant difference in the studied animals in Table 10.

Nutrition base prevalence and arthropod infestation

Animals observed with balanced diet intake were 260 out of which 20 (7%) animals were infested while 240 (92%) animals were non-infested. Animals having unbalanced diet were 240 out of which 180 (75%) animals were infested while 60 (33%) were non-infested (Table 11).

Arthropod infestation dominancy in ruminants

The observed animals showing tick infestation prevalence were 100, out of which 80 (85%) animals were infested while non-infested animals were 20 (14%).

The observed animals for lice infestation were 150, and the infested animals were 110 (73%) while the animals which not showed lice infestation were 40 (26). The total of 250 animals were observed for flea infestation and the infested animals were 160 (64%) while non-infested were 90 (36%) (Table 12).

Table 6. Treatment wise prevalence of insects

Treatment schedule	Yearly	6 Months	P(T<=t)	6 Months	3 Months	P(T<=t)	Total
Infested animals	70 (70%)	100(66.6%)	0.12	100 (66.6%)	160 (64%)	0.12	330 (66%)
Non –infested animals	30 (30%)	50 (33.3%)		50 (33.3%)	90 (36%)		170 (34%)
Total	100	150		150	250		500

Table 7. Seasonal prevalence of insects

Seasonal condition	Winter	Summer	P(T<=t)	Summer	Monsoon	P(T<=t)	Total
Infested animals	90 (64.2%)	100 (67%)	0.5	100 (67%)	130 (61%)	0.05	320 (64%)
Non-infested animals	50 (35.5%)	50 (33%)		50 (33%)	80 (38%)		180 (36%)
Total	140	150		150	210		500

Table 8. Insect infestation of animal hygiene management

Animal hygiene	Poor	Normal	P(T<=t)	Normal	Good	P(T<=t)	Total
Infested animals	70(77%)	6 (3%)	0.79	6 (3%)	54 (21.2%)	0.01	420 (84%)
Non-infested animals	20 (22%)	150 (96%)		150 (96%)	200 (78.7%)		80 (16%)
Total	90	156		156	254		500

Table 9. Nature of livestock and insect associations

Nature of livestock	Cows	Buffaloes	P(T<=t)	Buffaloes	Mix flock (Cow & Buffalos)	P(T<=t)	Total
Infested animals	180 (78%)	50 (62%)	0.4	50 (62%)	140 (73)	0.3	370 (74%)
Non-infested animals	50 (21%)	30 (37%)		30 (37%)	50 (26%)		130 (26%)
Total	230	80		80			190

Table 10. Feeding mode and insect infestation

Food-wise	Stall Feeding	Pasture Grazing	P(T<=t)	Pasture Grazing Mix	P(T<=t)	Total
Infested animals	15 (14%)	70 (58%)	0.9	70 (58%)	200 (53%) 0.3	265 (38%)
Non-infested animals	90 (85%)	50 (41%)		50 (41%)	75 (33%)	235 (18%)
Total	105	120		120	275	500

Table 11 Nutrition base prevalence of insect infestation

Nutrition	Balance	Unbalance	P(T<=t)	Total
Infested animals	20 (7%)	180 (75%)	0.96	200 (40%)
Non-infested animals	240 (92%)	60 (25%)		300 (60%)
Total	260	240		500

Table 12. Prevalence of insects in ruminants

Arthropods	Ticks	Lice	P(T<=t)	Lice	Fleas	Fleas	P(T<=t)	Total
Infested animals	80 (85%)	110 (73%)	0.12	110 (73%)	160 (64%)	160 (64%)	0.057	350 (43%)
Non-infested animals	20 (14%)	40 (26%)		40 (26%)	90 (36%)	90 (36%)		150 (56%)
Total	100	150		150	250	250		500

Discussion

Our study reports different types of arthropods associated with ruminants, such as lice species (*Pediculus sp*. and *Bovicola bovis* (Linnaeus, 1758)), tick species (*Hyalomma anatolicum* Koch, 1844, *Rhipicephalus microplus* (Canestrini, 1888) and *Haemaphysalis sulcata* Canestrini and Fanzago, 1877) and one flea species [*Ctenocephalus canis* (Curtis, 1826)]. The taxonomy of arthropods associated with large ruminants is indicated for the first time in the State of Azad Jammu and Kashmir.

The presence of *H. anatolicum* was reported in Azad Kashmir by Sultana et al. (2015). There is taxonomic record for identification lice species (*Pediculus* sp. and *B. bovis*) of Azad Jammu and Kashmir, but no literature is available about the presence of lice in association with ruminants. Moreover, the area where *H. anatolicum* was reported by Sultana et al. (2015) is a hot area, where the temperature in June rises to 50°C, but in the current study area the temperature never exceeds 20°C and winter temperature decreases even to –5° C. Our results about seasonal infestations match with the findings of Sultana et al. (2015).

The current findings indicate that females are more affected than males and similar results were found by Gebreselama et al. (2014). Our results show that adults are more prone to insects, so in comparison with the previous studies our study shows similar results as in Gebreselama et al. (2014), where adults were more infested than young animals due to greater browsing activities for greater food intake to have the necessary energy for reproduction.

Muhammad et al. (1999) observed that infestation was found more in adult animals. Animals older than 4 years (7.25%) were harder infested than those between 6 months to 2 years old (3.10%). Similar results were found by Kamani et al. (2010), who reported that adult animals are more infested than the young ones.

The presented findings indicate that insects' infestation rate is greater in cows than buffalos and similar findings were reported by Ghosh et al. (2007) because cows have dense hair which hides the insects. Buffalos have less hair and insects can be easily killed and/or detached by wavy action of switch. The current research indicates that arthropods` abundance increases in moon soon, followed by summer and winter; similar results have been reported by Iqbal & Nawaz (2007).

Our results indicate that arthropods` abundance decreases with good hygienic condition. Similar reports were found by Hartung (2005) who reported that good hygienic conditions protect farm animals from arthropod pests. In our findings, arthropod infestation was greater in mix herds feeding, followed by pasture grazing and stall feeding. Similar findings were reported by Kabir et al. (2011) who found that mixed herds pasture grazing causes more infestation than solitary and stall feeding. Balanced diet causes less arthropod infestation than unbalanced diet. This is also confirmed by the similar findings of Ghosh et al. (2007). Our results also indicate that fleas are dominant, followed by lice and ticks, similar to the results reported by (Drummond et al. 1981).

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