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Original Article

Parasitic prevalence in bat fauna captured from selected sites in northwestern Pakistan

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(With 3 figures)

Abstract

Present study was conducted to record ecotoparasitic prevalence in bat fauna of the northwestern parts of Pakistan. A total of 204 bat specimens representing 14 species were captured during a two year survey, extending from June 2015 through May 2016. A species of soft ticks *Argas vespertilionis* was identified from 23 bat specimens. Similarly, members of the family Dermanyssoidae (dermanyssoid mites) were isolated from 10 bat specimens, that of Spinturnicidae (spinturnicid mites) from 3 and Streblidae (bat flies) from 2 bat specimens. These parasites were collected using entomological tweezers and were identified on morphological basis. Further studies on parasitic prevalence, molecular characterization of bat parasites and their control measures are recommended.

Keywords: Argas vespertilionis, Dermanyssoidae, Spinturnicidae, haematophagous, chiroptera.

Prevalência parasitária na fauna de morcegos capturados em locais selecionados no noroeste do Paquistão

Resumo

O presente estudo foi realizado para registrar a prevalência de ectoparasitas na fauna de morcegos em partes do noroeste do Paquistão. Um total de 204 espécimes de morcegos, representando 14 espécies, foi capturado durante uma pesquisa de dois anos, de junho de 2015 a maio de 2016. A espécie de carrapato Argas vespertilionis foi identificada em 23 espécimes de morcegos. Da mesma forma, os membros da família Dermanyssidae (ácaros dermanyssoid) foram isolados de 10 espécimes de morcego, os da Spinturnicidae (ácaros spinturnicid), de 3, e os da Streblidae (mosca de morcego), de 2 espécimes de morcego. Esses parasitas foram coletados com pinça entomológica e identificados com base morfológica. Estudos adicionais sobre prevalência parasitária, caracterização molecular de parasitas de morcego e suas medidas de controle devem ser realizados.

Palavras-chave:Argas vespertilionis, Dermanyssidae, Spinturnicidae, hematófago, chiroptera.

1. Introduction

Almost all the mammalian species including bats have changed their distribution ranges while new distribution ranges are merely focused in Pakistan (Javid et al., 2012 a, b; Mahmood-ul-Hassan et al., 2011; Mahmood-ul-Hassan and Salim, 2015; Roberts 1977; Taber et al., 1967). Bats are important members of the terrestrial food chains and are distributed throughout the globe except some extremely colder parts of the world (Simmons 2005a, b). Similarly, the bat fauna of Pakistan is as diverse as any other region having similar climatic conditions (Mahmood-ul-Hassan et al., 2009).

Ectoparasites like monogeneans and most arthropods live on the outer surface of the body and affect majority of mammalian species including bats. However, their abundance and diversity especially in bats is not fully understood. Some parasites exist in hosts perennially while others affect only during critical stages of the host's lifecycle such as lactation or gestation and affect the development of juveniles and reproductive success (Bize et al., 2003; Lucan, 2006; Weaver and Aberton 2004). Hence these parasites reduce overall fitness of their hosts resulting in costs of host behavioral defenses (Hart, 1992). Animals overcome these costs by living in the habitats that are not suitable for parasites and such type of evidences of habitat selection as a defense against ectoparasites are well documented (Hart, 1992). Such types of selections might be for very short periods of time involving individual animals moving from locations with high to low ectoparasite abundance (Butler and Roper, 1996; Christe et al., 1994).

Bat ectoparasites like ticks and mite (arachnid species) is very much important issue to study. Among the number of Blood-sucking arachnids can not only play a vital role in ecological line but also in transmitting pathogenic, vector-borne diseases not among bats only, but also linking bats with domestic animals and humans (Klimpel & Mehlhorn, 2014; Ullah et al., 2019). Ticks are obligate ectoparasites and hematophagous vector agents that cause many diseases in wild as well as domestic animals round the globe (Bowman and Nuttall, 2008). Family Argasidae (soft ticks) is represented by nearly 200 species worldwide. These ticks are second to mosquitoes that affect humans as well as livestock globally (Labruna et al., 2014).

Ticks are further classified in to three main families, viz. family Argasidae (soft ticks), family Ixodidae (hard ticks) and family, Nuttalliellidae. These families have common basic characteristics depending on their behavior and life-style (Hoogstraal, 1985). Most argasids are fast feeders, ingesting relatively small amount of blood per meal and adult specimens can feed and reproduce repeatedly. They are highly resistant to starvation and can survive for several years without feeding in diapause period (Sonenshine, 1991; Dhooria, 2008).

Argas vespertilionis, round bat argasid generally occupies crevices in roosting site of the bats while their adults are rarely found on bats. Pipistrelles are the primary bat hosts of argasids. During feeding, these ticks insert their mouthparts in the body of the host (Hosseini-Chegeni and Tavakoli, 2013).

Family Spinturnicidae includes hematophagous mites and exclusively parasitizes bats. These mites show different degrees of specificity in relation to host families which may be due to ecology and geographical isolation of their hosts and life history strategies of parasites. Thus, the specificity is an important trait in the life history of the parasites. These mites have five stages of life cycle, the egg, larva, protonymph, deutonymph and adult. The egg and larval stages occur inside the pregnant females while nymph and adult mites are mostly found in patagium of infected bats (Almeida et al., 2015; Dusbábek and Los, 1968; Poulin and Mouillot, 2003; Wenzel and Tipton, 1966).

Members of the family Dermanyssidae are potential vectors. These parasites affect birds, bats and rodents and live inside their nests or in burrows. The mites are mostly well modified as they are capable of feeding rapidly on the blood of their host by using a special type of stylet or chelicerae which penetrates the epidermis. They possess the capacity for gorging themselves and are resistant to periods of fasting. Major public health problems are the transmission of diseases by these mites. Various authors have shown that these mites are concerned with the transmission of both bacterial infections (*Spirocheta, Salmonella*, *Pasteurella*, *Rickettsia*) and viral diseases (equine encephalitis viruses, fowl pox virus, west Nile virus, tick borne encephalitis viruses, the virus causing Newcastle diseases) and also aid in protozoanal and filarial transmission.

Bat flies are highly specialized ectoparasites and are associated with bats. Bat flies are divided into two families i.e. Nycteribiidae and Streblidae. Commonly bat flies reproduce viviparously in which eggs are fertilized internally and all larval stages develop within the female, nourished by special milk (Dittmar et al., 2006).

In Pakistan, there is extreme shortage of information regarding parasitic prevalence in bats. Present study was therefore planned to analyze the parasitic prevalence in bat fauna inhabiting northwestern parts of Pakistan.

2. Material and Methods

A survey to record the parasitic prevalence in bat fauna inhabiting northwestern Pakistan was conducted from June 2015 through May 2017. Bat specimens were collected using mist and hand nets from the selected sites and kept in separate cloth bags and habitat type, elevation, GPS coordinates, sex and specific locality of the captured specimens was recorded (Hamidullah, et al., 2019; Hamidullah et al., 2018; Javid et al., 2011; Rahman et al. 2015) (Table 1). All captured bats specimens were then euthanized, preserved in 70% ethanol and were transported to the laboratory of zoology university of Peshawar, Peshawar Pakistan for recording the bacular and cranial measurements of identification of species following (Salim, et al., 2016a, b; Shahbaz et al., 2014, 2015).

These specimens were kept in separate bat bags and were brought to the laboratory for parasitic analysis. The entire body of each bat specimen was fully inspected and parasites were collected through entomological tweezers. These parasites were preserved in 70% ethonal for further analysis (Zahn and Rupp, 2004).

Parasites were identified under microscope using descriptive morphological keys. These parasites were mounted on slide and sketches for larva and adults were drawn for morphometric and morphological analysis (Jones and Clifford, 1972; Kohls et al., 1969) and their prevalence (%) and mean tick intensity infestation was recorded using formula (1) and (2), respectively (Bush et al., 1997).

Table 1. GPS coordinates of the sampling sites explored during present survey.

Sampling Stations	GPS Location		1	2	3	4
	Latitude	Longitude				
MulakalyTuhaid Abad	N 34°47.074	E071°36.46	5♀	*M. muricola*	NC	815m
ShagoSalarzo	N 34° 49.105	E 071° 0.009	1♂	*P. tenius*	NC	720m
Tuhaid Abad	N34º 47.07	E71º 36.476	8(1♂,7♀)	*S. kuhlii*	NC	820m
JawerPayshat	N 34°53.508	E 071°31.714	3(1♂,2♀)	*M. formosus*	NC	658m
SayedBarkaly	N 34°55.276	E 071°40.914	4(1♂,3♀)	*P. cromandra*	NC	932m
Bazwany Lion cave	N 34°53.387	E 071°44.986	2(1♂,1♀)	*R. ferrumequinm*	EC	1812m
UopPeshawer	N 34°34.302	E 071°29.153	2(1♂,1♀)	*S. heathii*	NC	353m
UopPeshawer	N 34°34.302	E 071°29.153	10(2♂,8♀)	*P. tenius*	NC	353m
Jandool model school Toor	N 34° 57.104	E 071°31.207	9(3♂,6♀)	*P. cromandra*	NC	845m
ChalgazyPayshat	N 34°55.244	E 071°30.602	14(4♂,10♀)	*S. kuhlii*	NC	1345m
ChalgazyPayshat	N 34°55.244	E 071°30.602	4(2♂,2♀)	*P. cromandra*	NC	1345m
Swat FizagutZamurd Tunnel 1	N 34° 47.303	E 072°22.242	7(5♂,2♀)	*R. hipposiderous*	EC	923m
LardagyPayshat	N 34° 53.507	E 071°31.738	22(8♂,16♀)	*P. cromandra*	NC	954m
Swat Kabal	N 34° 41.896	E 071°20.345	3♀	*R. leschenaultii*	OC	1061m
Swat Fizagut	N 34° 47.321	E 072°22.197	4(3♂,1♀)	*P. tenius*	NC	955m
Swat khazakhelaJano	N 34° 65.602	E 072°29.512	7(4♂,3♀)	*S. kuhlii*	NC	1180m
Swat khazakhelaJano	N 34° 65.602	E 072°29.512	8(6♂,2♀)	*P. cromandra*	NC	1180m
Karaz masjid MundaDir	N 34° 34.510	E 071°40.933	2(1♂,1♀),	*S. kuhlii*	NC	835m
Karaz masjid MundaDir	N 34° 34.510	E 071°40.933	5(2♂,3♀),	*M. lyra*	NC	835m
Karaz masjid MundaDir	N 34° 34.510	E 071°40.933	5(1♂,4♀),	*M. lyra*	NC	835m
Camp masjid MundaDir	N 34° 34.518	E 071°40.950	13(6♂,7♀)	*S.kuhlii*	NC	835m
SumarbaghWadi Banda Dir	N 34°55.567	E071°34.54	7♀	*R. hipposiderous*	EC	1099m
Zoorbander Cave Zarabanda	N 34° 41.897	E 071°20.347	3♂	*R. microphyllum*	OC	1159m
PayshatBatkhela	N 34° 52.334	E 071°31.902	1♀	*M. muricola*	NC	968m
Zoorbander Cave Karbory	N 34° 41.703	E 071°19.840	11♂	*R. microphyllum*	OC	1141m
GDC Nawagi	N34º 41.896	E71º 20.345	7(1♂,6♀)	*P. javanicus*	NC	1031m
Raghan	N 34° 49.216	E 071°35.023	4(3♂,1♀)	*P. javanicus*	NC	800m
PayshatDormopaty	N 34° 52.149	E 071°31.827	27(8♂,19♀)	*P. cromandra*	NC	948 m
Mulakaly	N34º 51.324	E71º 41.812	1♂	*S. heathii*		859m
SumarbaghWadibanda	N 34°57.678	E071°39.12	4(2♂,2♀)	*M. muricola*	NC	1224m
GHSS Gardai	N34º 41.127	E71º 20.365	9(3♂,6♀)	*P. tenius*	NC	676m
Dary	N 34° 34.981	E071°36.545	2(1♂,1♀)	*S. heathii*	NC	848m
Dary	N 34° 34.981	E071°36.545	6(1♂,5♀)	*P. cromandra*	NC	848m

1 = Number and sex of captured bats, Male(♂), Female (♀), 2 = Species of the bats, 3 = Habitat type (NC = No cave dweller, OC = Opportunistic cave dweller, EC = Essentially cave dweller), 4 = Elevation of sampling stations.

Prevalence (%) = number of total infested bats x 100/ number of total examined bats (1)

Mean tick intensity infestation (MI) = total collected ticks/total infested bats (2)

3. Results and Discussion

Almost all the mammalian species including bats are infected by the parasites. These parasites might be seasonal or may infect the host during specific periods affecting health and well-being of the animals (Lourenço, 2008). In Pakistan, although few researchers have started focusing bats however, there is scanty of information regarding parasitic prevalence in bats. During present survey, a total of 204 specimens representing 14 bat species were captured from selected sites in northwestern parts of Pakistan. Out of 14 captured species, 9 species were infected by the parasites. These species included *Pipistrellus javanicus*, *P. coromandra*, *P. ceylonicus*, *Scotophiluskuhlii*, *S. heathii*, *Rhinolophus hipposiderous*, *Myotis formosus*, *Rhinopoma microphyllum* and *Rosettus leschenaultii*. Bats constitute one of the most diverse and well distributed groups of mammals and have adapted to live in close proximity to humans (De Blase, 1980; Roberts 1997). Hence, there is dire need to understand the parasites of bats and to find out their zoonotic importance. The parasites are transmitted either horizontally i.e. transferred from one individual to the other or vertically i.e. transferred from adults to juveniles. However, the horizontal transmissions are less harmful than vertical transmissions (Clayton and Tompkins, 1994).

Argas vespertilionis was formerly recognized as *Carios vespertilionis* but later it was identified as *Argas vespertilionis* (Figure 1) while dorsal side with

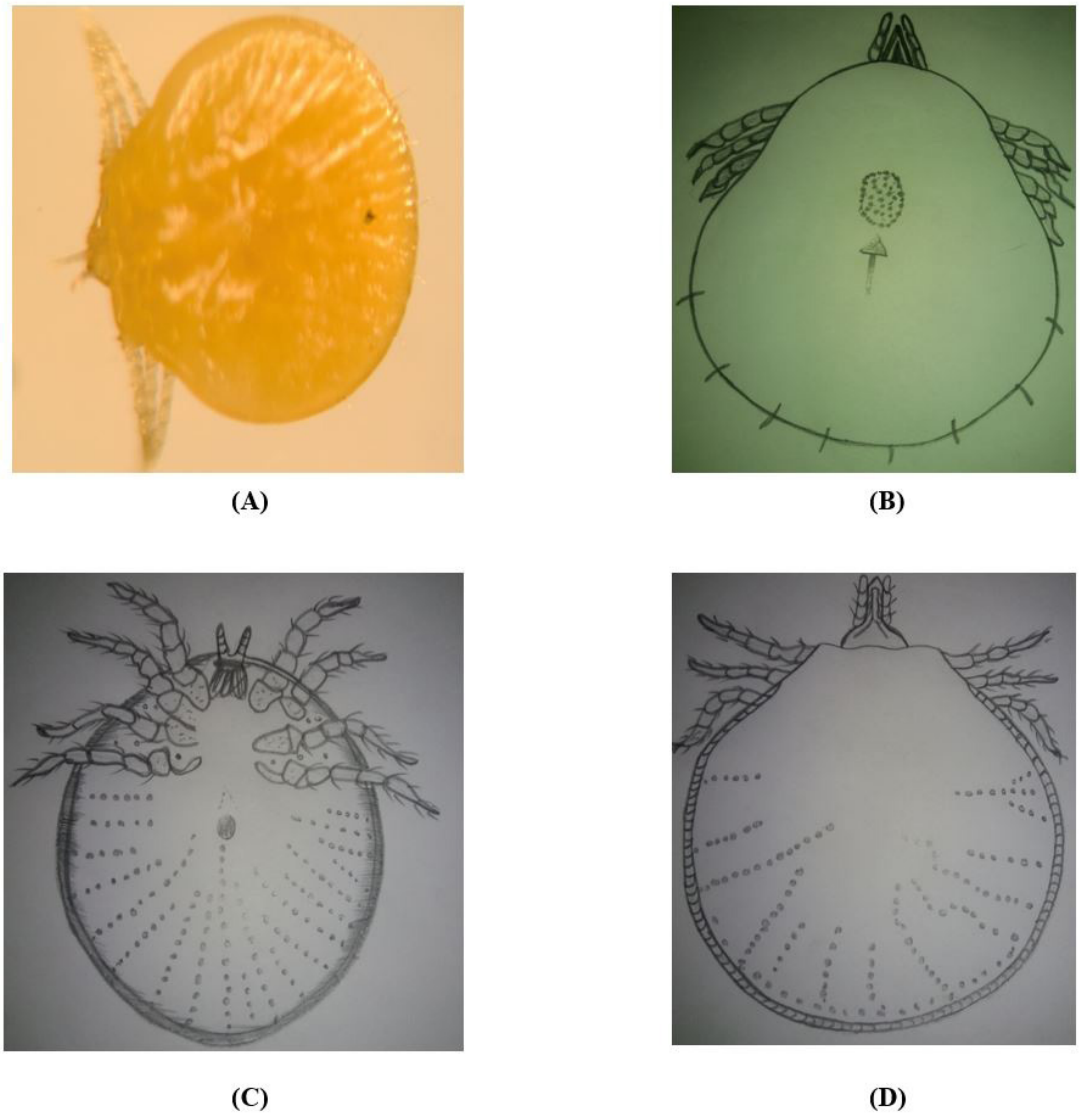


Figure 1. Photographs (A), drawing (B), ventral side and (C) dorsal side (D) of *Argas vespertitilionis*.

half appendages, hypostome, body margins, lateral view and dorsal view of tarsus have shown in (Figure 2 and 3). Genus *Argas* is represented by three species i.e. *A. boueti*, *A. confusus*, and *A.vespertilionis*. These congeners can parasitize same host and have closely related characteristics however can be differentiated from one another on the basis of their body shape. *A. vespertilionis* is a soft tick and also infect humans especially living in close proximity of the bat roosts (Hoogstraal, 1956). *A. vespertilionis* hosts *Nyctalus noctula, P. pipistrellus* and other bat species. In addition, these ticks also penetrate to houses and parasitize the humans (Gavrilovskaya, 2001). These ticks also have been reported from the houses and bed rooms of the people in France (Socolovschi et al., 2012). During present study, *A. vespertilionis* was observed as the most common parasite and was collected from 8 bat species. A total of 6 larvae of these soft ticks were collected from two infected *Pipistrellus javanicus* specimens with parasitic prevalence of 11.76% and mean parasitic intensity 3. Similarly, 15 larvae were collected from eleven *P. coromandra* specimens with parasitic Prevalence of 14.66% and mean parasitic intensity 1.36. Only one larva of *A. vespertilionis* was hosting *P. ceylonicus* specimen, parasitic prevalence was recorded 12.5% and mean parasitic intensity was one. Four specimens of *Scotophilus kuhlii* were hosted by 13 *A. vespertilionis* larvae with prevalence of 9.30% and mean parasitic intensity 3.25. Seven larvae were collected from single *S. heathii* specimen. The prevalence

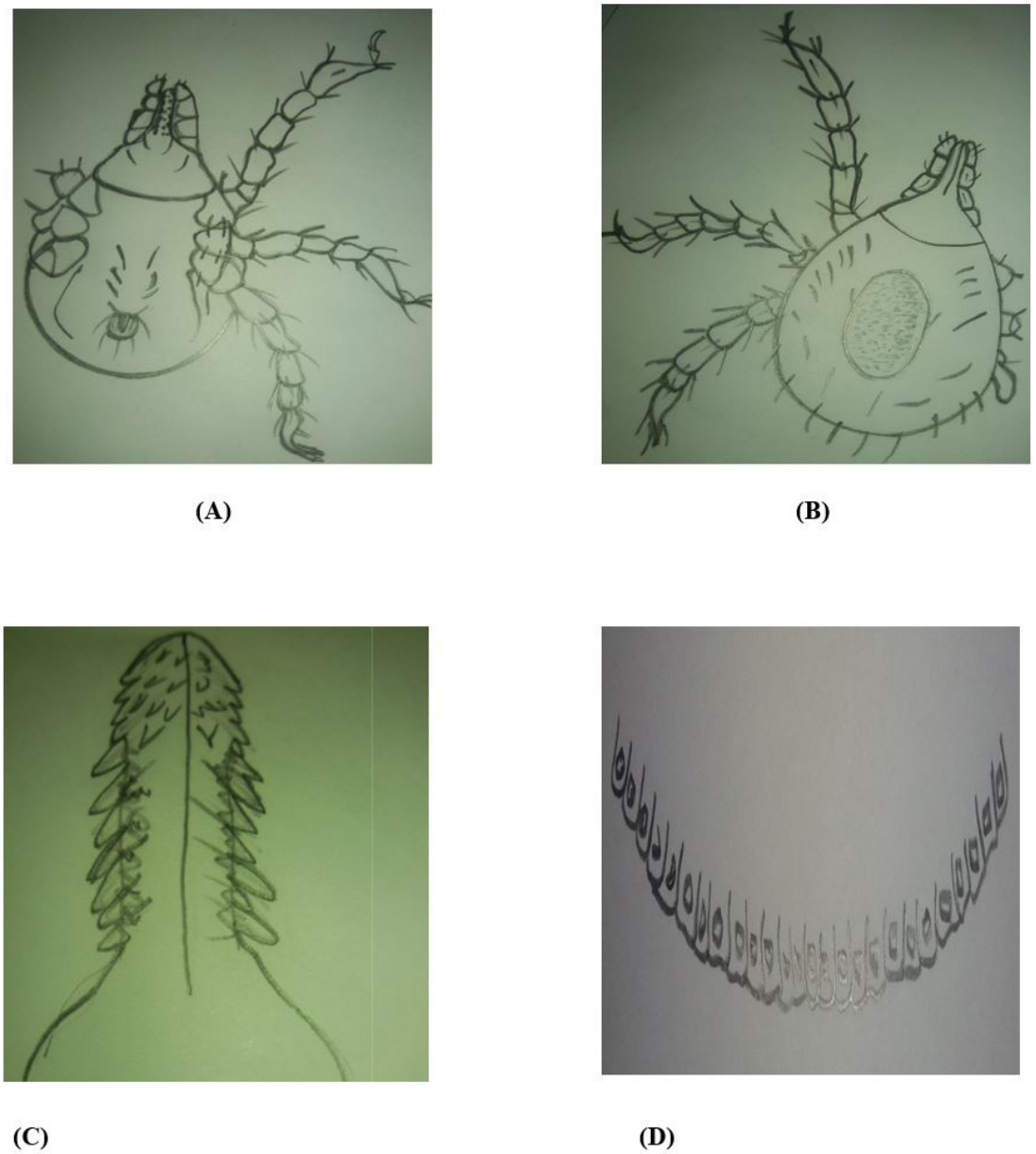
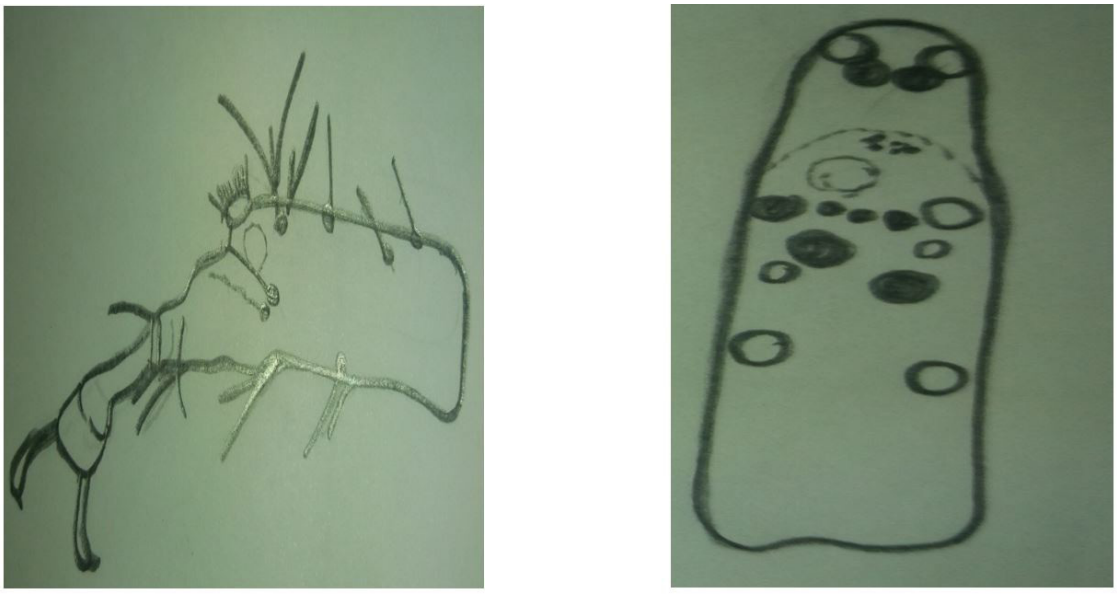


Figure 2. Ventral side (A), dorsal side with half appendages (B), hypostome (C) and (D) body margins of *Argas vespertitilionis*.



(A) **(B)**

Figure 3. Lateral view (A) and dorsal view (B) of tarsus of *Argas vespertitilionis*.

for *S. heathii*was 20% and mean parasitic intensity was recorded 7. Similarly, 2 *A. vespertilionis* larvae were isolated from single *Rhinolophus hipposiderous* specimen, the prevalence was 13.33% and mean parasitic intensity was 2. Only one larva of *A. vespertilionis* was collected from *Myotis formosus* specimen with prevalence of 33.33% and mean parasitic intensity one. Single larva of this soft tick was also isolated from *Rhinopoma microphyllum* specimen, with parasitic prevalence of 7.14% (Table 2).

Family Dermanyssoidea represents vast group of ubiquitous parasites and many of its members live in close relationship with rodents and birds, in their nests, burrows or on the surface of their hosts. Some species are hematophagous parasites while others are facultative parasites (Radovsk, 1985). The range of their hosts is very large and they can easily parasitize other species including farmed animals and humans, can survive periods of prolonged fasting, have ubiquitous characters and are resistant to climatic conditions. These characteristics make them interesting vectors for the dissemination of pathogens. During present study, out of 204 bat specimens captured during present survey, 10 individuals were hosted by dermanyssoid mites. Two larvae of these mites were collected from single *P. javanicus* specimen with percentage prevalence of 5.88% and mean parasitic intensity 2. Four larvae were collected from *P. coromandra* with parasitic prevalence of 5.33% and mean parasitic intensity 0.8. Similarly only one larva of Dermanyssoid mites was isolated from *P. ceylonicus* with percentage prevalence of 12.5% and mean parasitic intensity of 1. Three larvae of these mites were collected from *S. kuhlii* specimen with parasitic prevalence of 14.33% and mean parasitic intensity 1. One larva of Dermanyssoid mites was recorded from a single specimen of *R. hipposiderous*, the parasitic prevalence was 6.66% and mean parasitic intensity was recorded 0.5. Two larvae of Dermanyssoid mites were recorded from *M. formosus* with parasitic prevalence was 6.66% and mean parasitic intensity was 3.5. Similarly, single larva of these mites was isolated from *R. microphyllum* with parasitic prevalence of 60%.

Mites of the families Spinturnicidae and Argasidae are among the most frequently recorded ectoparasites of bats (Lucan, 2006). Spinturnicid mites are permanent, obligatory and host-specific ectoparasites of bats. Although spinturnicid mites spend their entire life cycle on bat wing membranes, they have been found in great numbers away from bats on the guano within the roost (Deunff and Beaucournu, 1981). Spinturnicid mites adjust their reproductive cycle to that of the host by massively infesting newborns, very vulnerable hosts (Christe et al., 2000). Newborns as well as juvenile bats are considered more attractive for parasites than adults because of less self-grooming proficiency (McLean and Speakman, 1997). During present study, a total of 6 larva of Spinturnicid mite were collected from 5 individual infested bats. From two *P. javanicus* specimens three larva of this mite species were collected with percentage parasitic Prevalence was 11.76% and mean parasitic intensity was 15, while 3 larva were collected from *S. kuhlii* with percentage parasitic Prevalence was 14.33% and mean parasitic intensity was 1.2 respectively.

Bat flies are highly specialized ectoparasites which are associated with bats. They get attached with the wing membranes of their hosts and feed on blood. These flies have

Table 2. Parasitic prevalence in bat fauna captured from selected sites in northwestern Pakistan.

Species of Bat	Argas vespertilionis		Dermanyssoid mites		Spinturnicid mite		Bat fly (Streblidae)	
	NEB/NIB	Prevalence	NEB/NIB	Prevalence	NEB/NIB	Prevalence	NEB/NIB	Prevalence
Pipistrellus pipistrellus	2/0	-	2/0	-	2/0	-	2/0	-
Pipistrellus javanicus	17/2	11.76	17/1	5.88	17/2	11.76	17/0	-
Pipistrellus coromandra	75/11	14.66	75/4	5.33	75/0	-	75/0	-
Pipistrellus tenuis	24/0	-	24/0	-	24/0	-	24/0	-
Pipistrellus ceylonicus	8/1	12.5	8/1	12.5	8/0	-	8/1	-
Scotophilus kuhlii	43/4	9.30	43/3	14.33	43/4	14.33	43/4	-
Scotophilus heathii	5/1	20	5/0	-	5/0	-	5/-	-
Rhinolophus ferrumequinum	2/0	-	2/0	-	2/0	-	2/0	-
Rhinolophus hipposiderous	15/1	6.66	15/1	6.66	15/0	-	15/0	-
Myotis muricola	10/0	-	10/0	-	10/0	-	10/0	-
Myotisf ormosus	3/1	33.33	3/2	66.66	3/1		3/0	-
Rhinopoma microphyllum	14/1	7.14	14/0	-	14/0	-	14/1	7.14
Megaderma lyra	5/0	-	5/0	-	5/0	-	5/0	-
Rosettus leschenaultia	5/0	-	5/0	-	5/0	-	5/1	20

NEB = Number of bat specimens examined, NIB = Number of infested bats.

wide distribution ranges and are represented by two families namely Streblidae and Nycteribiidae. Members of the family Streblidae usually parasitize bats (Dittmar et al., 2006). A total of 3 bat fly (Streblidae) were isolated from 2 infested bat species. From single *Rhinopoma microphyllum* specimen two bat fly of Streblidae family were collected with percentage parasitic Prevalence was 7.14% while 2fly of this species were collected from *R. leschenaultii* with percentage parasitic Prevalence was 60% and mean parasitic intensity was 2.

During present study, members of four families of ectoparasites were isolated from the body of the bats. Only one species *Argas vespertilionis* was exactly identified while three were identified up to family. This was the first attempt to identify parasites of bats in Pakistan and further studies are recommended for exact parasitic species identification in bats.

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