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ARTICLE

Seasonal variation in prevalence, parasite load and mean intensity of ectoparasites in *Pipistrellus kuhlii* (Chiroptera: Vespertilionidae) from Iran

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ABSTRACT We studied quantitatively the seasonal variation in prevalence, parasite load, and mean intensity of two species of ectoparasites in free ranging populations of Kuhl's bat, *Pipistrellus kuhlii* in western Iran. In total, 348 live bats (230 males and 118 females) were collected using mist net during May to November 2013. All bats identified as to sex and the number of ectoparasite species in each bat was counted. On 348 *P. kuhlii* captured, 5355 ectoparasites were observed. Two species of ectoparasites belonging to *Argas vespertilionis* (Argasidae) and *Steatonyssus* sp. (Macronyssidae) were identified. In late May and at the beginning of June, the peaks of ectoparasite load in bats were recorded and then dropped during June to November. The number of ectoparasites in pregnant female bats in May-June during pregnancy was significantly higher than non-pregnant females ($p \leq 0.05$). A significant correlation was found between ectoparasite load and the ratio of body mass to the length of forearm (W/FA), as an index of body condition, indicating that parasite load has apparent impact on bat's health. Our findings indicated that parasite loads correlate with season, sex and reproductive condition of the host.
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Introduction

Ectoparasites live and feed on external surfaces of its host and are present on almost all species of mammals, including bats. Bats are one of the most diverse mammals and harbor numerous ectoparasites (Moura et al. 2003) which are themselves parts of the biodiversity. They are, also, dependent on the host to complete their own lifecycle (Sharifi et al. 2008, 2013). Studies on various groups of vertebrates have shown that ectoparasites influence host fitness (Fenner and Bull 2008), body condition and reproductive success (Neuhaus 2003), as they can cause mortality, morbidity, reduce fecundity or regulate host population size and change demographic characteristics (Tomás et al. 2007). Therefore, it is important to understand ectoparasites species richness, abundance, and life history in order to recognize the potential impact on host fitness (Lourenço and Palmeirim 2007). Species richness and diversity of bat ectoparasite assemblages may be related to several factors including, home range, behavior, size and roost type of the host (Zhang et al. 2010; Webber et al. 2015).

Studies on host-parasite relationships in free living bat species shows that ectoparasitic load appears to vary among male and female hosts. Most studies found that males are more parasitized than females (Morand et al. 2004, Amo et al. 2005). In contrast, study on five species of bats showed that all ectoparasites were more prevalent on female than male hosts (Christe et al. 2007). This may be due to the congregation of hundreds of individuals in many species of female bats in maternity colonies that despite thermoregulatory benefits, adult females and young may face high rates of ectoparasite exposure (Czenze and Broders 2011). There are several important biological differences between males and females that may explain sex biased parasite load. Differences in sex-specific behaviors such as aggressive behavior between males for mating may increase the likelihood of becoming more infected and cause different exposure to parasites (Krasnov et al. 2005). Also, negative association between steroid hormones, particularly testosterone, and immune responses are thought to play a major role in the difference between male and female ectoparasite load (Roberts et al. 2004)

There are studies that have reported the relationship between the number of parasites and the condition of bat health (Christe et al. 2000; Sharifi et al. 2008, 2013). In some bats, ectoparasites reproduction events are synchronized with host reproduction, so the number

Table 1. Average monthly number of bats, number of parasites, prevalence (%), parasite load (Mean ± SE) and intensity in male (M) and female (F) of *P. kuhlii*.

Month	No. of bats (M)	No. of bats (F)	No. of parasites (M)	No. of parasites (F)	Prevalence (M)	Prevalence (F)	Parasite load (M)	Parasite load (F)	Intensity (M)	Intensity (F)
May	56	28	1505	1319	100	100	26.87 ± 4.44	47.10 ± 10.62	26.87	47.10
June	55	38	829	507	47.27	84.21	15.07 ± 1.86	13.34 ± 3.71	31.88	15.84
July	29	15	31	238	93.10	80	1.06 ± 2.05	15.86 ± 2.59	1.14	19.83
August	21	12	80	231	42.85	83.33	3.80 ± 1.34	19.25 ± 2.43	8.88	23.10
September	29	10	93	113	75.86	100	3.20 ± 1.50	11.30 ± 2.39	4.22	11.30
October	19	6	101	69	52.63	100	5.31 ± 1.34	11.50 ± 2.46	10.10	11.50
November	21	9	180	59	52.38	77.77	8.57 ± 1.83	6.55 ± 2.08	16.36	8.42

of ectoparasites is highest in pregnant and lactating bats and after the parturition the peak of parasite abundance belong to juveniles (Christe et al. 2000; Bartonicka and Gaisler 2007; Lourenço 2008). The effect of variation in the intensities of a parasitic mite (*Spinturnix myoti*) in relation to the reproductive cycle and immunocompetence of its bat host (*Myotis myotis*) has shown that pregnant females were less immunocompetent and harbored more parasites than non-reproductive females (Christe et al. 2000). There are also studies that demonstrate differences in the social contact between different segments of a species population, as well as, differences in roosting habits among bat species, populations, and sexes, can cause differences in parasite load (Lourenço 2008; Sharifi et al. 2008, 2013; Postawa and Szubert-Kruszyńska 2014).

Kuhl's pipistrelle, *P. kuhlii* (Kuhl 1817) is a small bat with a circum-Mediterranean area distribution occurring in entire southern Europe. In Iran, this bat occurs both in Mesopotamian semi-deserts and in mountainous regions of the Zagros Range (Benda et al. 2006). In the present study, we summarize seasonal variation in infestation, prevalence, parasite load and intensity of the ectoparasitic in Kuhl's pipistrelle, *P. kuhlii* in western Iran. We also aim to explore the relationship between parasite load and ratio of body mass to forearm length (W/FA) as an indicator of health condition.

Materials and Methods

Kuhl's pipistrelle *P. kuhlii* (Kuhl 1817) used in this study were mist-netted in western Iran (34° 23' N, 47° 07 'E) during May to November 2013. Immediately following capture, bats were placed in individual cloth bags (30 × 50 cm). The following data were recorded: date, sex, weight (g), forearm length (mm), reproductive stage for females by palpation (pregnant, non-pregnant) (Sharifi et al. 2008) and the number of ectoparasite specimens on the wing membranes of each individual bat, as well as the ventral and dorsal parts of the body. We spent approximately 20 seconds searching each bat for ectoparasites by blowing on their pelage. Parasites were collected using a pair of stainless steel pointed tweezers and were preserved in 1.5 ml Eppendorf tubes with 70% ethanol. The sampled parasites were identified using available resource (Stanyukovich 1997; Radovsky 2010; Hosseini-Chegeni and Tavakoli 2013).

Prevalence of parasite is expressed as the percentage of parasitized individuals, relative density or parasite load as mean number of ectoparasites per bat and intensity of parasitism as the mean number of parasites found on parasitized individuals separately for male and female. The significance of patterns of prevalence parasite among male and female of *P. kuhlii* were analyzed using Fisher's exact test. Parasite load was compared using an ANOVA and infestation intensity was compared using *t*-tests. Bat health was indicated by the ratio of body mass to forearm length (Sharifi et al. 2008) and was also compared to parasite load using the Pearson correlation coefficient (Miller 2014). The statistical program package SPSS (v. 16) (spss.software.informer.com) was used for all analyses.

Results

In total, 348 bats (230 males and 118 females) were examined for ectoparasites during May to November 2013. One species of tick (*Argas vespertilionis*, Argasidae) and one species of mite (*Steatonyssus* sp., Macronyssidae) were identified as ectoparasites on *P. kuhlii*. The mite parasites did not identify in species level due to the morphological complexity. Of the total of 5355 ectoparasites that were observed in this study, 502 were belonging to *Argas vespertilionis* and 4853 to *Steatonyssus sp*. The overall prevalence of ectoparasites on all bats was 73.48 ± 6.11%. Table 1 shows the number of bats, number of parasites, parasite load, prevalence and intensity of infestation in male and female of *P. kuhlii*.

Average prevalence of total infestation on male and female bats was 66.29 ± 8.77% and 89.33 ± 3.85%, respec-

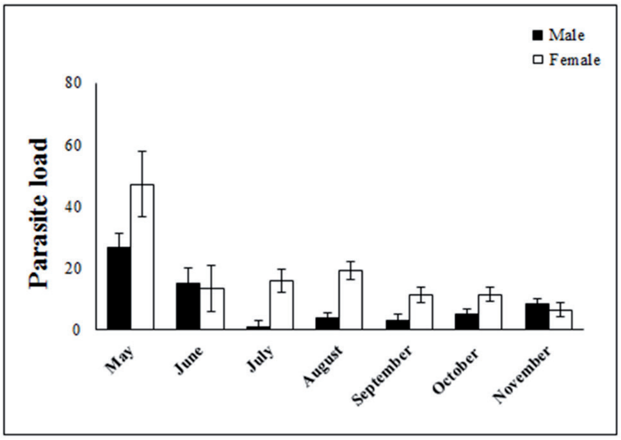

Figure 1. Variation in parasite load for males and females *P. kuhlii* in May to November 2013.

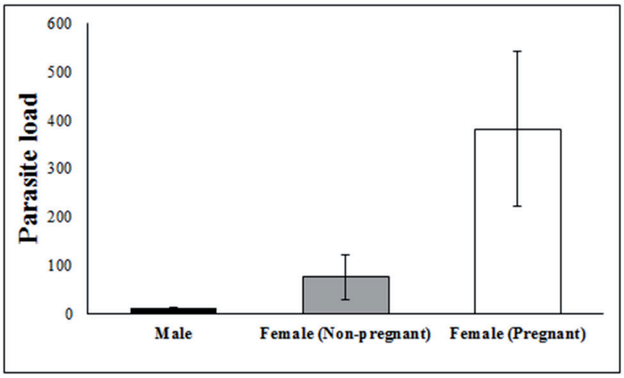

Figure 2. Total parasite load in male, non-pregnant and pregnant females of *P. kuhlii*.

tively. Prevalence was significantly different between males and females of *P. kuhlii* ($p = 0.04$). Parasite loads for males and females were 9.12 ± 3.42 and 17.84 ± 5.10 and mean intensities of infestation were 14.20 ± 4.34 and 19.58 ± 4.98, respectively. A peak of ectoparasite load recorded in May and then decrease was found until the end of November (Fig. 1). Parasite load was not significantly different between males and females of *P. kuhlii* ($p = 0.18$). Parasite intensity was not significantly different between males and females of *P. kuhlii* ($p = 0.43$). During the pregnancy until the parturitions in mid-June, a significant difference was recorded in the number of ectoparasites between pregnant females, non-pregnant females ($p < 0.05$) (Fig. 2). There was a significant relationship between parasite load and the health indicator of adult *P. kuhlii* ($p = 0.001$); (male: $p = 0.001$; female: $p = 0.13$).

Discussion

The present study provides new data regarding prevalence, intensity and parasite load in a free-living *P. kuhlii* during May to November. Average peak value for ectoparasite load was in May when the parturition had not started. Following parturition in early June a sudden decrease is seen in the ectoparasite (Table 1, and Fig. 1) in male and female *P. kuhlii.* A similar reduction in prevalence and average intensity began in June. This rapid change in the amount of ectoparasite load is the result of changes in life history traits which causes differences in the social contact between different segments of a population that can eventually cause differences in parasite load. There are many studies that support the differences in the social contact as well as differences in roosting habits that cause differences in parasite load in the temperate bats that experience a long-term winter hibernation, characterized by a rapid emergence from winter hibernacula, the formation of maternity colonies and parturition in late spring (Altizer et al. 2003; Sharifi et al. 2008, 2013; Webber et al. 2015).

Ectoparasite loads varied seasonally, declining from spring to winter. In this study, the pick of ectoparasite infestation in males and females observed in May. This is in consistent with other studies. Sharifi et al. (2008) found that the number of *Spintunix* sp. increased in June and July and decreased towards August. Observation of reproductive system in *P. kuhlii* by Barak and Tom-Tov (1991) demonstrated that hundreds of female bats aggregate at maternity colonies at the beginning of March. They reported similar group of males (7-16 individuals) in roosting sits which tend to stay in same group for 3 months. Hemmati et al. (2000) showed that in Iran *P. kuhlii* give birth in late of June. The higher host population density of bats in spring could be an important factor explaining distribution and abundance of parasites and cause of the species to face higher parasite loads (Poulin and Morand 2000; Stanko et al. 2002). Direct physical contact can exchange permanent parasites when bat species aggregate during mating or foraging (Altizer et al. 2003; Webber et al. 2015). Ticks and mite are flightless and permanent ectoparasites and host transfer depends on close contact of bats (Mathison and Pritt 2014). Age of the colony and colony size are also known to have an influence on levels of ectoparasite infestation on bats (Lučan 2006).

Our study revealed that average ectoparasite infestation was higher in females than in males, resulting in a higher parasite load in the population in spring and summer. Consistent with this study, greater prevalence and intensity of ectoparasites were found in females of little brown bats (*Myotis lucifugus*) compared to male bats (Czenze and Broders 2011; Webber et al. 2015). A similar result has also been observed between ectoparasite load in male and female of bat fly parasites on some Phyllostomid bats in southeastern Brazil (Komeno and Linhares 1999). Results of studies conducted by Dick et al. (2003) showed that relative density of ectoparasite load in several female bats from central Pennsylvania was higher than males. In a study on grooming behavior and parasite load in the greater horseshoe bat (*Rhinolophus ferrumequinum*), a significant difference between parasite load of male and female have been reported (Wohland 2000). It has been shown by several authors that females of most temperate zone bat species usually aggregate during the breeding season in nursery colonies, whereas males are mainly solitary or in small groups and occupy satellite roosts (Lewis 1995). The effect of such colonial habits of female bats during reproduction in favor of parasite transmission has also been revealed (Christe et al. 2000). Moreover, high temperature of nursery roost sites, including the heat generated by tight associations among individuals in a colonial cluster, may also favor parasites' reproductive output (Marshall 1981). These two factors could lead to a strong sex-linked difference in the opportunity to become infested (Christ et al. 2007). Therefore, sex bias in parasite rate most likely seems from sex-specific differences in host social behavior, with female aggregation during the reproductive period that play a central role for the evolution of this pattern.

Most biologists assume that ectoparasites on bats can influence the behavior and health of their hosts and may act as vectors for disease or cause physical damage to their hosts (Marshall 1982). However, there are other reports that have documented that ectoparasite load has no effect on health condition of host (Sharifi et al. 2008, 2013). In conclusion, in our study there was a significant relationship between parasite load and body condition of adult *P. kuhlii*.

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