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Exploring bat parasites in selected caves of Fars Province, Southern Iran

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Abstract

This study aimed to document the significance of parasites in transmitting zoonotic pathogens from bats to domestic animals and humans. To achieve this, we performed an examination of ten large caves in Fars Province (Iran), which served as bat roosts. Bats were captured using a mist net and identified based on morphological characteristics. The ectoparasites were collected using brushes and preserved in separate vials containing 70% ethyl alcohol. Bats were released after collecting all ectoparasites from their bodies, except 3 bats. The collected ectoparasites were then prepared as slides and identified using a taxonomic key. The results revealed that out of 66 bats examined, 56 individuals were infested by a total of 113 ectoparasites. These included bat flies [*Nycteribia kolenatii* (22.1%), *Paratrichobius* sp. (10.6%) and *Penicillidia* sp. (11.5%)], mites [*Macronyssus flavus* (55.0%)], and ticks [*Ixodes* sp. (0.8%)]. Among the bat species, *Myotis blythii* and *Rousettus aegyptiacus* exhibited the highest levels of ectoparasite infestation. Three bats from different species were transferred to the lab, dissected, and their feces were centrifuged using the Formalin Detergent method. Several worms, including nematodes [*Seuratum* sp.] and Cestoda [*Hymenolepis rhinopomae*], were isolated from their intestines and feces. The identified specimens were properly deposited in the Iranian National Parasitology Museum (University of Tehran). Some of these findings represent new records in Iran. The results indicate a high infestation rate with bat flies showing a wide distribution and high density in the research region. Therefore, further research is recommended to expand our knowledge of bat parasites.

Key words: Bat, bat fly, mite, Nematoda, roost, tick, zoonotic pathogen

Introduction

Bats constitute 21 families and more than 1450 species, and are the second largest group of mammals (Mammal Diversity Database, 2024). Their bodies provide a suitable habitat for various parasites. Despite their positive roles in the ecosystem, bats are known to serve as reservoirs or vectors for certain zoonotic diseases (Sándor et al., 2019). Numerous arthropod species have been identified as bat parasites (Amarga et al., 2017; Burgin et al., 2018). Among these ectoparasites are fleas (Ischnopsyllidae), bat flies (Nycteribiidae and Streblidae), mites (Spinturnicidae), and bugs (Cimicidae), which all inhabit the fur of bats (Orlova et al., 2021; 2022), potentially leading to infections (Zabashta et al., 2019; Léger, 2020).

Bat flies, true flies belonging to the dipteran families Nycteribiidae and Streblidae, are exclusive blood-feeding ectoparasites that target bats (Reeves and Lloyd, 2019). They possess specialized claws adapted for clinging to bats and are parasitic over a wide range of bat species (Szentiványi et al., 2019).

Ticks on the other hand, act as vectors for several diseases, and are medically significant arthropods (Hornok et al., 2019). Both hard ticks such as *Ixodes* species and soft ticks (Argasidae) exhibit specializations for bats (Sándor et al., 2019). *Ixodes* ticks have a global distribution, with some species like *I. simplex* showing a particular preference for specific host species (Holz et al., 2018).

Previous research has demonstrated that bats harbor a wide variety of endoparasites and ectoparasites. Despite increasing global attention on bat ectoparasites, there is limited available data in Iran. Two species of ticks were reported, including *Argas vespertilionis* by Hosseini-Chegeni and Tavakoli (2013) and *Ixodes vespertilionis* by Vatandoost et al. (2010). Other reports included a nematode and bat fly (Hemmati et al., 2013), mite (Yousefi et al., 2021), and more parasites by Malek Hosseini et al. (2017). Information on bat endoparasites in Iran is even scarcer than for ectoparasites (Ghasemi et al., 2016). Notably, Kazemirad et al. (2020), isolated *Lecithodendrium* sp. and *Castoria* sp. from the intestines of two species belonging to the genus *Rhinopoma.* Additionally, it is essential to consider that many pathogenic microorganisms, including bacteria (Hornok et al., 2019; Lee et al., 2021), fungi (Haelewaters et al., 2018), and viruses (Temmam et al., 2022), are parasitic or symbiotic with bat parasites. These microorganisms can potentially have adverse effects on host fertility and survival. Consequently, bat parasites can directly impact their hosts and indirectly contribute to the spread of pathogenic microorganisms (Hornok et al., 2019; Sándor et al., 2019).

Fars Province has an area of 133,000 km^2 and is in the southwest of Iran between 50°36' to 55°35' E and 27°03' to 31°40' N. The existence of different climate regimes, including cold winters, temperate winters, and warm and dry summers, the extent of coverage plants, the presence of rivers, mountains, caves and cracks in this region provide suitable habitats for a large and diverse population of bats (Ghasemi, 2019). The caves in this Province are the main roosts for at least 24 of the 50 species of bat known from Iran (Shahabi et al., 2017). Despite the significant diversity and large number of bats in this region, there is little information about their parasites. Given the epidemiological significance of bat parasite species, the present study was conducted to enhance our understanding of these parasites and their geographical distribution in this region.

Material and Methods

This research was conducted between spring and the end of summer season in various caves located in 3 climatic regions in Fars Province (Table 1, Fig. 1). Within this region, 10 large caves were deliberately chosen, and all sampling activities were conducted with the approval of the Department of Environmental Protection (permission number: 400/14585). Bats were captured using a mist net, and were carefully placed in individual cloth bags. Subsequently, the bats were identified using taxonomic keys at the site (Benda et al., 2012) from Czech Republic, and later verified by Professor Benda. The sex and primary morphometric data of each bat were meticulously recorded. The furred body of them and their bags were examined at the capture site or the lab.

The ectoparasites found on the bats, including flies, mites, and ticks, were removed using forceps or toothbrushes with utmost care. To facilitate observation, they were viewed through a magnifying glass or stereomicroscope. Each collected ectoparasite was carefully separated from the individual bat and preserved in separate vials containing a solution of 70% ethyl alcohol and 5% glycerin 5%, for further observation. The parasitic samples were cleared in lactophenol, for at least 48 hours. Following this, the specimens were soaked in a 10% KOH solution overnight, washed in two changes of tap- water, and dehydrated using an alcohol series (30%, 50%, 75%, 90 and 100%). Subsequently, the specimens were mounted in glycerine gelatin on a glass slides with coverslips, embedded, and scanned for taxonomic identification (Theodor, 1967). Permanent slides were also prepared for the museum specimen collection. These slides were observed under the loop at 4x zoom and photographed using a PC-digital camera. Taxonomic identification was carried out according to literature and taxonomic keys.

Three different species of bats (*Rousettus aegyptiacus*, *Myotis blythii*, and *Rhinolophus ferrumequinum*) were collected and sent to the lab for examination. Throughout this process, ethical considerations were carefully adhered to in handling the animals. Their intestines, gall bladder, liver, and spleen were meticulously examined, and certain worms were isolated from their intestines. To facilitate egg observation, feces were removed from the worms and centrifuged using the formalin-detergent sedimentation method. Subsequently, the worms were washed in a 0.8% saline medium, and fixed in alcohol (70%). The cestodes were pressed carefully between two slides and then fixed with 4% formalin for 24 hours. These specimens were further stored in alcohol (70%). To clarify the cuticle and observe detailed structures, the cestodes were cleared in lactophenol and then mounted in glycerin. Identification of the worms was based on diagnostic keys and morphological characteristics, including the total length of the worm, lip features, type of esophagus (size and presence of esophageal bubbles), shape and size of spicules, mating sac, and other superficial structures such as teeth, spines, and papillae on the body (Yamaguti, 1961; Angoma et al., 2020). All identified specimens were properly deposited in the Iranian National Parasitology Museum (University of Tehran) for future reference and research. Additionally, one tick was isolated from *R. ferrumequinum*. The identification of the bat hosts were confirmed by Dr. Vahid Akmali from Iran and Professor Benda from Czech Republic.

Results

The survey revealed the presence of a diverse bat fauna, sampling a total of 66 individuals belonging to 9 species from 7 genera and 7 families (Table 2, Fig. 2).

Some of these results are the first records of bat parasite in the southern Iran. Almost all bats were infected with parasites and all caves, bat roosts, showed contamination. According to the obtained results, *R. aegyptiacus* and *M. blythii* were host a greater variety of parasites. The female bats were more infested than male, and large caves showed more parasitic infection. Among the identified ectoparasites, *N. kolenatii* belonging to the family Nycteribiidae (Fig. 3), was the most abundant species in this study. A significant number of mites, including the identified species *Macronyssus flavus* were observed on the inner surface of wings and tail plates; however, these mites were not specifically identified. While contamination with bat flies was observed in all caves surveyed, there were variations in species richness and the parasite - host relationship between the caves. The infestation levels for a particular bat species were relatively similar in all caves.

Table 1: Characteristics of the examined caves, Fars Province, Iran.

Cave (capture site)	Longitude (E)	Latitude (N)	Elevation (m)		Location	Climate
1	Dalkhoon	52˚05'42.08"	30˚14'16.50"	2200	Sepidan	Cool
2	Kan Gohar	53˚54'30.70"	30˚18'03.33"	2170	Bavanat	Cool
3	Balazar	52˚01'13.00"	31˚09'39.00"	1500	Shiraz	Cool-temperate
4	Plangan	52˚44'01"	30˚03'31.00"	1941	Marvdasht	Temperate
5	Gohardan	53˚16'14.05"	29˚57'54.05"	1878	Arsanjan	Temperate
6	Eshkaft Shitan	53˚13'58.00"	28˚34'08.00"	1043	Meymand-Jahrom	Warm
7	Shahpoor	51˚36'42.38"	29˚48'27.75"	1320	Kazerun	Warm
8	Sangtrashan	53˚34'46.99"	28˚29'08 .20"	1090	Juyom	Warm
9	Shafagh	52˚56'58"	28˚40'14.00"	1530	Firuzabad	Warm
10	Bonwo	53˚53'53.00"	27˚22'58.00"	955	Lar	Too warm

Figure 1: The examined caves in Fars Province. The numbers (1–10) show the examined caves.

Table 2: The identified bats in examined caves, Fars Province, Iran.

Family	Genus/ Species
Pteropodidae	1- *Rousettus aegyptiacus* (Geoffroy, 1810)
Rhinopomatidae	2- *Rhinopoma hardwickii* Gray, 1831
	3- *Rhinopoma microphyllum* (Brünnich, 1782)
Hipposideridae	4- *Asellia tridens* (Geoffroy, 1813)
Rhinolophidae	5- *Rhinolophus euryale* Blasius, 1853
	6- *Rhinolophus ferrumequinum* (Schreber, 1774)
Vespertilionidae	7- *Myotis blythii* Tomes, 1857
Miniopteridae	8- *Miniopterus pallidus* Thomas, 1907
Emballonuridae	9- *Taphozous nudiventris* Cretzschmar, 1830

Figure 2: The identified bat species in the examined caves of Fars Province, Iran.

Table 3: The identified bat parasites in the examined caves of Fars Province, Iran.

Parasite	Genus/species	ID*	Figure
Ectoparasite (Bat fly)	1- *Penicillidia* sp. Kolenati, 1863	810	2
	2- *Nycteribia kolenatii* Theodor and Moscona, 1954	812	3
	3- *Paratrichobius* sp. (Costa Lima, 1921)	813	4
Ectoparasite (Mite)	4- *Macronyssus flavus* (Kolenati, 1857)	814	5
Ectoparasite (Tick)	5- *Ixodes* sp. Latreille, 1795		6
Endoparasite (Nematoda)	6- *Seuratum* sp. Hall, 1916	811	7
Endoparasite (Cestoda)	7- *Hymenolepis rhinopomae*	815	8

*ID: The record number of identified species in the Iranian National Parasitology Museum.

This wingless fly exhibits a wide thorax and an elongated abdomen. It possesses long legs, especially the hind legs, and each has curved claws (Fig. 3A). The head features reduced compound eyes, and the antennae are densely located between them (Fig. 3B). The abdomen is adorned with numerous setae (Fig. 3C). *Rousettus aegyptiacus*, *Rhinolophus ferrumequinum*, and *Asellia tridens* are hosts for this species.

This species, *Nycteribia kolenatii*, showcases a red to light brown body color with an elongated abdomen. The head boasts large compound eyes (Fig. 4A). Notably, the first abdominal segment has a row of compact hairs, and two-pronged claws are present at the end of the legs (Fig. 4B). An important distinguishing feature is the presence of a ctenidium, a comb-like structure consisting of thick spines used for secure purchase to the bat's hair, on both the abdomen and mesothorax (Fig. 4C).

Individuals of *Paratrichobius* were not identified to species. *Paratrichobius* sp. has 2 paired fully developed wings (Fig. 5A) and numerous spines on its legs (Fig. 5B). The hind legs are longer than others (Fig. 5C). Pairs of heavy setae are observed on tergum and lateral and ventral margins of the abdomen (Fig. 5C). *R. hardwickii*, *R. microphyllum*, *Rousettus aegyptiacus*, *Taphozous nudiventris*, and *M. blythii* are hosts for this species.

This mite species has an elongate white body with long legs, each equipped with terminal nails (Fig. 6A). Its size ranges from 1 to 2 millimeters, and they are challenging to separate from the skin. A large dorsal shield, covering with setae is a prominent feature. The first pair of long legs is inclined towards the front (Fig. 6B). The palps are needle-like, and the chelicera lacks teeth. This spesie belonging to Macronyssidae family was seen on *R. hardwickii*, *R. microphyllum*, *Miniopterus pallidus*, and *M. blythii.*

A female *Ixodes* tick was found on the dorsum of *R. ferrumequinum*. Its body is oval, and length is 6.5 mm. Its abdomen was soft and full of egg. Characteristics used in identification include: the dorsal shield covers half of its dorsum (Fig. 7A). It has no eyes but its mouthparts and club- shaped pedipalps are long (Fig. 7B). The genital aperture is positioned at the end of the cephalothorax and internal spur reverse the first legs (Fig. 7B), and it has the U-shaped anal groove which arches anterior from the anus (Fig. 7C).

This specimen belongs to Phylum Nematoda, Order Spirurida, Family Seuratidae, and Genus *Seuratum.* The body is cylindrical with 19.2 mm in length and 0.42 mm diameters. Transverse lines are seen on the external surface of body (Fig. 8A). It has lips, two cervical papillae, two amphid and lateral cerebral ganglions at the anterior end (Fig. 8B). This species is female due to the presence of valve and uterus and absence of spicule at posterior end. *Rousettus aegyptiacus* and *M. blythii* are hosts for this species of endoparasite.

Figure 3: *Penicillida* sp., (A) whole mount, (B) anterior end and (C) posterior end.

Figure 4: *Nycteribia kolenatii*, (A) whole mount, (B) anterior view and (C) posterior view of body.

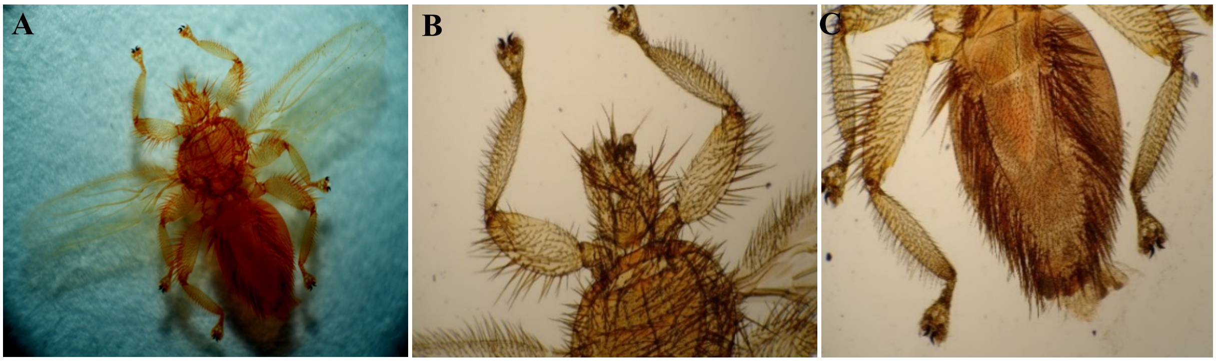

Figure 5: *Paratrichobius* sp., (A) whole mount, (B) anterior view and (C) posterior view of body.

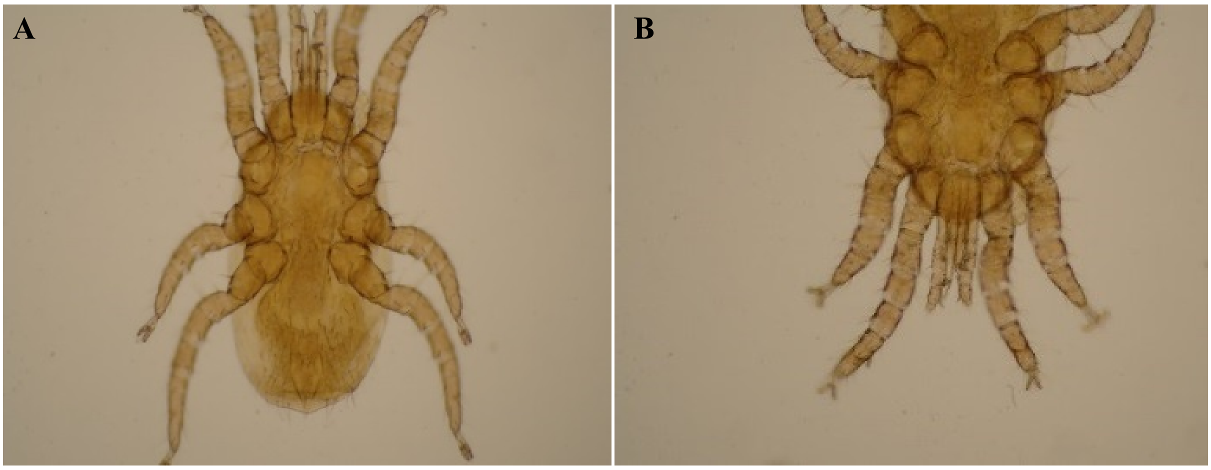

Figure 6: *Macronyssus flavus*, (A) ventral view (40X) and (B) anterior part of the ventral view (40X).

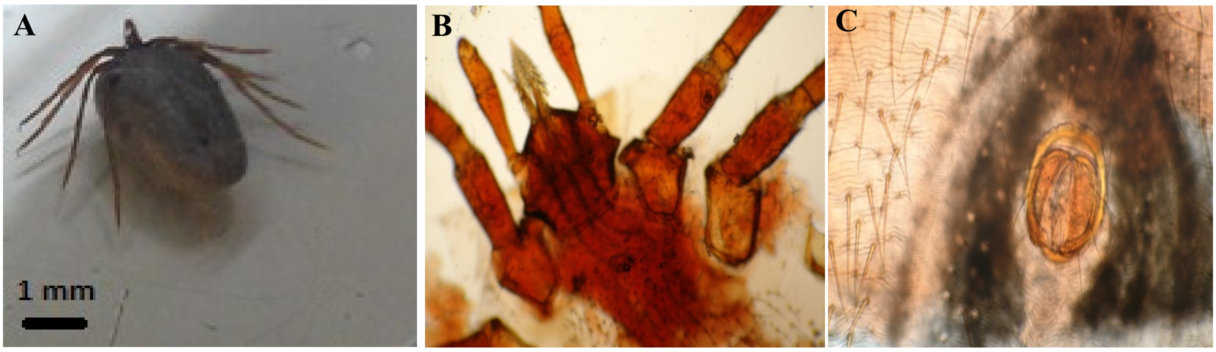

Figure 7: *Ixodes* sp., (A) whole mount (4X), (B) anterior end (100X) and (C) posterior end (100X).

Figure 8: *Seuratum* sp., (A) anterior end, (B) posterior end, and (C) mid body.

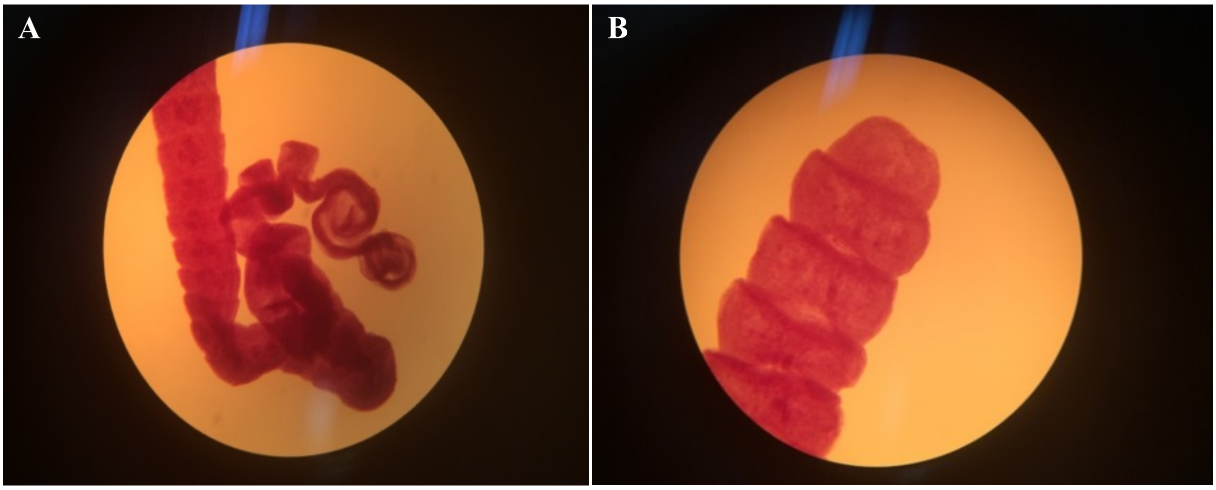

Figure 9: *Hymenolepis rhinopoma*, (A) whole mount (10X) and (B) anterior end (40X).

Discussion

Despite the high population and wide distribution of bats in Fars Province (Ghassemi, 2019), there is a scarcity of literature concerning the identification of bat parasites in Iran. In this study, we selected a number of caves in different climatic regions of Fars Province, and observed that the most bats and the largest number of parasites were in the temperate and warm climate regions. Bats generally select roosting sites with moderate temperatures, as this strategy lessens the energy expenditure required for thermoregulation, especially during reproductive periods or early ontogeny (Johnson and Lacki, 2014; Fabianek et al., 2015). However, these same conditions create a suitable environment for the reproduction and proliferation of arthropod pests. The present results show that the numerous caves and favorable climates in this region serve as excellent habitats for bats and their parasites (Kaka Abdella and Ghasemi, 2017; Shahabi et al., 2017; Akmali et al., 2019). The combination of these factors contributes to the prevalence of bats in the region, making it a favorable location for the study of bat parasites and their interactions with their hosts.

Ectoparasitic arthropods, acting as vectors of zoonotic pathogens, can cause illnesses in mammals such as bats. Several factors play a crucial role in determining the suitability of bats as parasite hosts, including their body size, body temperature, and the density of fur covering their bodies. In the context of Fars Province, the research region, the warm climate and stable temperatures within the caves facilitate the population growth and spread of these parasites. While the limited number of samples prevents a comprehensive analysis of parasite prevalence, the data obtained in the present study suggests that larger caves and larger host bats tend to have a higher density of parasites. These large caves, characterized by a greater population and diversity of bats (Ghassemi, 2019), provide ideal conditions for parasite survival and reproduction. The timing of bat surveys at the end of spring to summer is crucial, as it corresponds to the peak populations of both parasites and hosts (bats). This research documented that multiple bat species were infested by *Nycteribia* sp. and *Penicillidia* sp. This finding is in line with the observations of Ramasindrazana et al. (2017) and Verrett et al. (2022).

Paratrichobius dunni was reported on *M. blythii* by Vatandoost et al. (2010), and our finding aligns with this research, indicating that this parasite prefers larger bats. Similarly, *Penicillidia* sp. been reported on *M. capaccinii* in Fars Province (Benda et al., 2012), and *Penicillida jenynsii* has been found on *Miniopterus schreibersi*, *Miniopterus fuliginosus*, *Myotis macrodactylus* and *Rhinolophus ferrumequinum* (Kim et al., 2012; Lee et al., 2021). The family Streblidae is known to be an obligate bat ectoparasite, and Borkent et al. (2018) have recorded a total of 239 species with significant morphological variations. Within the *Paratrichobius* genus, six species have been described (Wenzel, 1976; Hernández-Arciga et al., 2016), but in this research, the specific species could not be identified, indicating that molecular diagnosis is recommended. *Paratrichobius* species are generally considered to be host-specific, typically parasitizing only one host species (Dick and Patterson, 2007), but the observations from this research, indicate that it was found on three out of nine bat species studied. This suggests that it don't have a species-specific host preference.

The higher number of parasites found on the bodies of fruit bats compared to insectivorous bats may be related to their diet (Wenzel, 1976). However, other factors such as the size of the bat's body, the characteristics of their roosts, and the population density of bats in a particular area also play a significant role in determining the parasite population (Borkent et al., 2018).

In addition to the previously mentioned species, the other identified bat fly species belong to the Nycteribiidae. *Penicillidia conspicua* and *Penicillidia dufourii* have been previously reported on *Miniopterus schreibersii* (Malek Hosseini et al.,

2016) and *M. blythii* (Hemmati et al., 2013). The results of the current study reveal that three other bat species are also hosts for these fly species, indicating that these parasites have a wide range of hosts. Furthermore, the existence of different bat species in the same cave environment might facilitate the transmission of the parasite from one host to another. The presence of the numerous mites on both small bats like *Rhinopoma hardwickii*, and larger bats like *R. aegyptiacus*, highlights the broad spectrum of infection caused by this parasite, consistent with findings from previous research (Orlova et al., 2021; 2022).

The observed tick has been identified as a female of *Ixodes*. According to the taxonomic key, its scutum form, U-shaped anal groove, and their mouthparts which protrude forward identify it as *Ixodes ricinus.* The presence of this species has been reported as ectoparasite of domestic mammals in the northern and western regions of Iran (Salari Lak et al., 2008; Vahedi-Noori et al., 2012). Prior research had suggested the range extension of the European *I. ricinus* into the north of Iran (Naddaf et al., 2020). The observation of only one specimen, *I. ricinus*, on the horseshoe bat in this research may be due to bat's ability to fly and migration. Balazar cave, the capture site of this species, is 54 km west of Shiraz, and was created as a result of water erosion. It is in a cool-temperate enviroment, and a stream always flows in it, so it is permanently humid. Sándor et al. (2019) documented 4 species of *Ixodes* as species parasitizing bats with varying degrees of host specialization. The species *I. ariadnae*, *I. simplex*, and *I. vespertilionis* are known as parasites of bats in Iran (Vatandoost et al., 2010; Malek Hosseini et al., 2016), but *I. ricinus* had not previously been recorded. Molecular analysis is needed for definitive species identification. These findings add to our understanding of the distribution and prevalence of ticks among bat populations in Iran.

The prevalence of mites on the bats in most examined caves indicates high infestation with this ectoparasite. Large bat colonies and short distance between them, make it difficult to detect a specific host, and a precise molecular method is needed for identifying them. Malek-Hosseini et al. (2016) reported *Macronyssus granulosus* on the body of *Miniopterus pallidus* in Yasuje, and the presence of *Spinturnix psi* and *S. myoti* on *M. pallidus* and *M. blythii* were reported by Yosefi et al. (2021). The present research identified *Rhinopoma hardwickii* and *R. microphyllum* in addition to the *Myotis* species as hosts for *Macronyssus flavus*.

Hymenolepis rhinopomae was previously reported from *M. blythii* by Hemmati et al. (2013). However, in the current study, the observation of this worm in the gut of 2 out of 3 bat species suggests a high infection rate with this parasite. The presence of large bat guano in the cave environment can facilitate the spread of the infection, as it provides a suitable environment for the parasites to thrive and spread.

Conclusion

All the examined caves in Fars Province harbored parasites, although the specific prevalence and intensity of ectoparasites were not quantified. Among the parasites observed, flies were the most abundant, and larger bats, such as *M. blythii* and *R. aegyptiacus*, showed the highest levels of infestation. Considering the rich diversity of bat fauna in Iran, especially in Fars Province, it would be expected to host a wide variety of parasites. Additionally, the ability of bats to fly enables the dispersion of these ectoparasites, which might contribute to their prevalence in different cave environments. Therefore, there is a strong recommendation for conducting an extensive study of bat parasites in this area to gain a more comprehensive understanding of the parasite diversity, prevalence, and their potential impact on bat populations and the ecosystem. Such research can also aid in implementing appropriate management and conservation strategies for bat populations in the region.

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Author contributions

Farangis Ghasemi: The sample collection, performing laboratory operations and article writing; Mohammad Miri: Field and laboratory operations.

Conflict of interest

The authors declare that there are no conflicting issues related to this research article.

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