Faunal distribution of fleas and their blood-feeding preferences using enzyme-linked immunosorbent assays from farm animals and human shelters in a new rural region of southern Iran

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Abstract Blood sucking insects, such as fleas, are responsible for the transmission of many infectious disease-causing agents which impose an intolerable burden on the health of people living particularly in endemic parts of the world. Fleas (Insecta: Siphonaptera) are found in many parts of the world including Iran. Both adult male and female fleas are obligatory ectoparasites. They are one of the main public health concerns as a result of their nuisance or the potential to act as vectors of a number of medically-important pathogens. The current study was conducted to examine the geographical distribution and fauna of fleas and their anthropophagic index in part of Fars province, southern Iran. This study was

the first to be done in Iran. A total of 20 villages were randomly selected. From October 2011 to May 2012, adult fleas were collected by direct hand catch from human to animal shelters. Overall 848 fleas, most of which were blood-fed, were captured from the floor or the body of farm animal hosts (cattle, sheep, goat and hens). Only two different genera of fleas were identified, the main species (99.76 %) was human flea, *Pulex irritans*. The village of Shamsabad was the most heavily infested area. *P. irritans* had an anthropophagic index of 15 % using indirect enzyme-linked immunosorbent assays (ELISA). It could be concluded that *P. irritans* is widely distributed in this area. Based on their blood feeding activity, fleas thus posed a serious health threat to residents and their economically important livestock in this part of Iran.

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Introduction

Some of the most devastating infectious diseases, such as plague, are transmitted by insect vectors and cause a dramatic health burden to people living in endemic regions of the world (Eisen et al. 2006). Patients suffering from plague are typically affected by the sudden onset of fever, chills, weakness and headache which could be confused with the clinical signs from other endemic diseases (Moemenbellah-Fard et al. 2012). The blood sucking insects, such as fleas (Siphonaptera: Pulicidae), have always coexisted with their human hosts (McElroy et al. 2010). They are usually found near human dwellings in many parts of the world. Adult fleas are obligatory blood sucking ectoparasites of mammals and birds, the majority of them being exclusive blood vessel-feeders (Lehane 2005). They are one of the main public

health concerns as a result of their nuisance or the potential to act as vectors of a number of medically-important pathogens (Bitam et al. 2010; Eisen and Gage 2012). Different populations of fleas have different roles in pathogen transmission in various parts of the world (Krasnov 2008). Fleas feeding on man and his domesticated animals usually transmit pathogens of a number of diseases like murine typhus.

It is generally envisaged that the dispersal of parasitic insects is determined by the distribution of their vertebrate hosts, which, in turn, is influenced by their environment (Bush et al. 2001). Most parasites are affected by environment not only through hosts, but also by direct environmental factors. This is particularly demonstrated by ectoparasitic arthropods which show a wide range of host-parasite relationships; from permanent attachment on the host (e.g. lice) to sporadic probes on a host (e.g. mosquitoes) and are thus exposed to various environmental effects. Fleas are in a middle situation in this range, alternating between periods on the host body and periods being off the host. So the habitat of a flea is defined as its host in a particular habitat (Krasnov et al. 2002).

Flea infestations are naturally restricted to those hosts which rest, for example, in man-made wooden shelters such as cattle sheds, sheep pens, or other animal lairs, since this could provide optimal environmental conditions for the completion of their life cycle (Traub 1985). They spend much of their time off-host, consequently they are known as floor fleas (Yin et al. 2011), or periodic ectoparasites (Lehane 2005). Most fleas infest their host temporarily then pass to another of the same kind, while others move from one to a different host species. There are reports of flea infestations on economically valuable livestock including cattle (Heukelbach et al. 2004), sheep (Sertse and Wossene 2007), goats (Christodoulouopoulos et al. 2006) and other ruminants (Kaal et al. 2006; McCauley et al. 2008).

As a result of public complaints from local residents on flea bites, this study was carried out to determine the faunal distribution of fleas in a new rural region of southern Iran. Apart from the study by Shoorijeh et al. (2008) which was conducted on dogs in part of Fars province, no investigation has been located by authors on fleas in this part of Iran. This study was thus conducted to investigate and identify the geographical distribution of fleas' fauna and their ELISA-based anthrophilic feeding preferences from farm animal sheds and human dwellings in Marvdasht county, Fars province, southern Iran.

Materials and methods

Study sites

This study was focused in Marvdasht County of Fars province (29°–30°N, 52°–53°E) which lies in south central

parts of Iran (Fig. 1). It covers an area of 125000 km² which is 7.6 % of all Iranian territory. Its population density was 35 per km² mostly (61 %) located in urban areas and about 29 % of whom were 0–14 year old in 2006. Its mean annual temperature is 18 °C, mean annual precipitation about 300 mm and altitudinal range of 900–1630 m above sea level. The main occupation of people who live in this rural area is agriculture and farming.

Study design

This study was conducted in a descriptive analytical way to determine the fauna and distribution of fleas feeding on humans and animals from the start of October 2011 to the end of May 2012. The study population included all male and female adult fleas and hosts (e.g. sheep, goat, cattle and hen) in the rural areas of Marvdasht. The inclusion criteria were host age of at least 2 months up to 1 year upper age limit, presence of fleas (only adult males and females) confirmed by study stuff, and indoor shelters. The exclusion criteria were outdoors, those hosts kept outside shelters, hosts dipped in insecticide-treated baths, and immature stages (eggs, larvae or pupae) of fleas. As no prior planning were set for summer, the data were restricted to the autumn, winter and spring of the study years. The sample size was randomly selected from among all rural ruminant breeding places. In order to select the sample size, a clustered sampling method was done. On this basis, Marvdasht area was divided into five regions: north, south, west, east and center. Using the table of random numbers, a total number of 20 county villages (Table 1) were randomly considered as sampling places from each of these regions (Fig. 1). The selection of sheds, rooms and hosts were also conducted randomly. An equal amount of time (30 min) was spent in each search activity.

Adult male and female fleas were collected from different parts of human and animal dwellings by direct hand catch method using a hand lens with ×10 magnification. The fleas were identified by morphological characteristics using standard taxonomic keys. They were then segregated according to sex and species of fleas, place and topography, type of dwellings, type of host, location on host, age and number of hosts, and their anthrophagic index.

Study samples

All fleas in the randomly selected groups were examined by health officers under the supervision of a medical entomologist. The trial was conducted in accordance with local laws and regulations based on global guidelines.

Enzyme-linked immunosorbent assay (ELISA)

A randomly-selected sample size of 200 fleas were subjected to a capture ELISA (Sarkari et al. 2008) after

Fig. 1 Map of the 20 study villages (A Koohsabz; B Ghasemabad; C Booreki; D Shamsabad; E Tajabad; F Nasrabad; G Fathabad; H Firoozi; J Jahanabad; J Mahjenabad; K Khaniman; L Golijan; M Maragolu; N Dashtak; O Barezabad; P Hassanabad; Q Taleghani; R Bizjanolia; S Baniyeke; T Sidan) in Marvdasht county of Fars province in southern Iran

Table 1 The geographical distribution of flea species in relation to their hosts' frequency in different villages of Marvdasht County, Fars province, southern Iran

Village	Flea spp.	Gender (%)			Hosts (%)			
		Female	Male	Total	Sheep	Goat	Hen	Calf
Koohsabz	<i>P. irritans</i>	26 (3.07)	18 (2.12)	44 (5.19)	100 (5.99)	50 (2.99)	35 (2.09)	–
Ghasemabad-Ramjerd	<i>P. irritans</i>	24 (2.83)	14 (1.65)	38 (4.48)	80 (4.79)	60 (3.59)	20 (1.20)	–
Booreki	<i>P. irritans</i>	28 (3.30)	18 (2.12)	46 (5.42)	55 (3.29)	25 (1.50)	30 (1.80)	–
Shamsabad	<i>P. irritans</i>	23 (2.71)	27 (3.18)	50 (5.89)	4 (0.24)	6 (0.36)	–	–
Tajabad	<i>P. irritans</i>	25 (2.95)	13 (1.53)	38 (4.48)	22 (1.32)	18 (1.08)	20 (1.20)	–
Nasrabad	<i>P. irritans</i>	26 (3.07)	14 (1.65)	40 (4.72)	110 (6.59)	60 (3.59)	35 (2.09)	–
Fathabad	<i>P. irritans</i>	26 (3.07)	12 (1.41)	38 (4.48)	115 (6.89)	85 (5.09)	24 (1.44)	–
Firoozi	<i>P. irritans</i>	21 (2.48)	14 (1.65)	35 (4.13)	–	–	–	96 (5.75)
	<i>C. canis</i>	–	1 (0.12)	1 (0.12)				
Jahanabad	<i>P. irritans</i>	20 (2.36)	24 (2.83)	44 (5.19)	3 (0.18)	3 (0.18)	–	–
Mahjenabad	<i>P. irritans</i>	22 (2.59)	22 (2.59)	44 (5.19)	75 (4.49)	42 (2.51)	40 (2.4)	–
Khaniman	<i>P. irritans</i>	13 (1.53)	35 (4.13)	48 (5.66)	–	–	–	10 (0.6)
Golijan	<i>P. irritans</i>	21 (2.48)	23 (2.71)	44 (5.19)	–	–	–	6 (0.36)
Maragolu	<i>P. irritans</i>	16 (1.89)	22 (2.59)	38 (4.48)	–	–	–	4 (0.24)
Dashtak	<i>P. irritans</i>	22 (2.59)	18 (2.12)	40 (4.72)	50 (2.99)	30 (1.80)	22 (1.32)	–
Barezabad	<i>P. irritans</i>	21 (2.48)	25 (2.95)	46 (5.42)	4 (0.24)	4 (0.24)	–	–
Hassanabad	<i>P. irritans</i>	15 (1.77)	25 (2.95)	40 (4.72)	60 (3.59)	30 (1.80)	–	–
Taleghani	<i>P. irritans</i>	19 (2.24)	24 (2.83)	43 (5.07)	–	–	–	35 (2.09)
	<i>C. canis</i>	–	1 (0.12)	1 (0.12)				
Bizjanolia	<i>P. irritans</i>	20 (2.36)	20 (2.36)	40 (4.72)	36 (2.16)	32 (1.92)	–	–
Baniyeke	<i>P. irritans</i>	17 (2.00)	25 (2.95)	42 (4.95)	4 (0.24)	6 (0.36)	–	–
Sidan	<i>P. irritans</i>	29 (3.42)	19 (2.24)	48 (5.66)	80 (4.79)	44 (2.63)	–	–
Total	–	434 (51.18)	414 (48.82)	848 (100)	798 (47.78)	495 (29.64)	226 (13.53)	151 (9.04)

removing part of the blood from their midgut on to the standard Whatman filter paper. The blood spot on each filter paper was punched and blood was eluted from the paper by overnight incubation of each spot in wells with 100 µl of phosphate-buffered saline (PBS).

Antibody-capture ELISA, was carried out in flat bottom 96-well microplates (Nunc, Nalgel, Nunc, International, Roskilde, Denmark). Microplates were coated with 100 µl of anti-human immunoglobulin (5 µg/ml) in coating buffer (0.05 M carbonate-bicarbonate buffer, pH 9.6) and incubated at 4 °C overnight. Plates were washed 5 times in phosphate-buffered saline-Tween 20 (PBST, pH 7.4 containing 0.05 % Tween 20). Blocking was done, using 3 % skim milk in PBST for 2 h. Plates were washed as before and 100 µl of fleas blood samples, eluted from Whatman filter paper, was applied to the plates and incubated for 1.5 h at room temperature. The plates were washed as before and 100 µl of horseradish peroxidase conjugated anti-human immunoglobulin (Sigma) at a 1/2000 dilution in PBST was added and incubated for 1 h at room temperature. After washing as before, the plates were incubated with chromogen/substrate (100 µl/well of OPD, 0.025 % H₂O₂ in 0.1 M citrate buffer, pH 5) and after 30 min the absorbance at 490 nm was measured using an ELISA microplate reader.

Data analysis

Data on host age, geographical status of residence (rural), date, topography, infestation season, genus, species and sex of flea, location of flea on host, blood-feed status and type of infestation were recorded and compared using Student's *t*-tests. The data entry and analysis were conducted using SPSS (version 16) software. The differences in proportions for the included variables were compared using χ^2 test. A *P* value of ≤ 0.05 was considered indicative of a statistically significant difference.

Results

From the study area, a total of 848 adult fleas were collected from different parts of Marvdasht County (Table 1). From these fleas, only 750 male and female fleas were partially or fully blood-fed. Only two different genera were identified. A clear majority of these ($N = 846$, 99.76 %) were *Pulex irritans*, while *Ctenocephalides canis* was represented by only 0.24 %. Adult males of *P. irritans* were mostly found in Khaniman ($N = 35$, 4.13 %), while females were more frequent in Sidan ($N = 29$, 3.42 %). No overall sex-biased differences were noticed among the study locations. The Shamsabad village had the highest percentage ($N = 50$, 5.89 %) of male and female fleas, followed by Sidan and Khaniman ($N = 48$, 5.66 % each).

Among five large ruminant (calf) breeding regions no other hosts were located, and vice versa (*i.e.* among 15 small ruminant -sheep/goat- breeding regions no cattle was found). Overall, 1670 hosts including sheep, goat, hen and cattle were searched for the presence of fleas. The village of Shamsabad was the most affected area by fleas among all the study locations. In a few villages where ≤ 10 small ruminant hosts lived, there were higher proportions of adult fleas present on these hosts, indicating their likely general vulnerability to such heavy infestations.

Fleas were mostly (67 %) found on hilly areas than on plateaus. Most fleas ($N = 536$, 63 %) were caught from the floor of animal shelters (Table 2). There was a significant difference between the frequency of floor fleas and that of host fleas ($P < 0.05$). Their presence was more pronounced in animal sheds than in human houses. The most frequent floor fleas ($N = 48$, 5.66 %) were found in Sidan, where small ruminant hosts were not infrequent ($N = 124$, 7.42 %); whilst the most frequent host fleas were discovered in Shamsabad with several emaciated small ruminant hosts ($N = 10$, 0.6 %). Moreover, the hands and trunk had the minimal and maximal levels of flea infestations ($N = 6$, 155; 6 and 18 %), respectively.

In Marvdasht, *P. irritans* had the highest activity in spring and its lowest activity was in winter. Their anthrophilic index was found by indirect ELISA test to be 15 % (Table 3). Fleas from the village of Taleghani with no small ruminant hosts had the highest (6 %) proportion of human blood in their gut, whereas fleas with the minimal value for ELISA-positivity test came from the village of Tajabad with a median number of small ruminant hosts.

Discussion

In the present study, only two species of fleas, *P. irritans* and *C. canis*, were collected from the floor and on the body of farm animal hosts. The former species was considered the most prevalent pulicid vector in this study area. The results of the present study confirmed that human flea, *P. irritans*, was widely distributed in all parts of Marvdasht County. It was mostly associated with animal dwellings. It appeared that host-associated blood factors such as skin thickness, hair density and depth of superficial blood vessels could make certain places more suitable for flea infestation (Krasnov 2008). The anthropophagic index (15 %) of human flea in this study showed that it posed a relative risk to the public health of residents in this region. As stated before, fleas have the ability to switch their hosts swiftly and indiscriminately (Eisen and Gage 2012; Krasnov et al. 2012). This is very important in the light of their ability to transmit a number of life threatening pathogenic agents such as plague and murine typhus.

Table 2 The distribution of floor fleas and host fleas in different villages of Marvdasht County of Fars province, southern Iran

Village	Flea spp.	Floor fleas number (%)	Host fleas (%)			
			Head & Hands	Trunk	Neck	Feet
Koohsabz	<i>P. irritans</i>	44 (5.19)	–	–	–	–
Ghasemabad-Ramjerd	<i>P. irritans</i>	38 (4.48)	–	–	–	–
Booreki	<i>P. irritans</i>	46 (5.42)	–	–	–	–
Shamsabad	<i>P. irritans</i>	–	24 (2.83)	22 (2.59)	2 (0.24)	2 (0.24)
Tajabad	<i>P. irritans</i>	38 (4.48)	–	–	–	–
Nasrabad	<i>P. irritans</i>	40 (4.72)	–	–	–	–
Fathabad	<i>P. irritans</i>	38 (4.48)	–	–	–	–
Firoozi	<i>P. irritans</i>	35 (4.13)	–	–	–	–
	<i>C. canis</i>	1.0 (0.12)				
Jahanabad	<i>P. irritans</i>	–	21 (2.48)	20 (2.36)	1 (0.12)	2 (0.24)
Mahjenabad	<i>P. irritans</i>	44 (5.19)	–	–	–	–
Khaniman	<i>P. irritans</i>	–	28 (3.30)	20 (2.36)	–	–
Golijan	<i>P. irritans</i>	–	21 (2.48)	20 (2.36)	2 (0.24)	1 (0.12)
Maragolu	<i>P. irritans</i>	–	16 (1.89)	22 (2.59)	–	–
Dashtak	<i>P. irritans</i>	40 (4.72)	–	–	–	–
Barezabad	<i>P. irritans</i>	–	16 (1.89)	27 (3.18)	1 (0.12)	2 (0.24)
Hassanabad	<i>P. irritans</i>	40 (4.72)	–	–	–	–
Taleghani	<i>P. irritans</i>	43 (5.07)	–	–	–	–
	<i>C. canis</i>	1.0 (0.12)				
Bizjanolia	<i>P. irritans</i>	40 (4.72)	–	–	–	–
Baniyeke	<i>P. irritans</i>	–	18 (2.12)	24 (2.83)	–	–
Sidan	<i>P. irritans</i>	48 (5.66)	–	–	–	–
Total	–	536 (63.21)	144 (16.98)	155 (18.28)	6 (0.71)	7 (0.82)

Previous studies have reported the presence/absence of herbivores such as sheep, goat and cattle could affect the number of fleas in an area (McCauley et al. 2008). This might have an effective repercussion on the human health risk in zoonotic diseases. Moreover, man-made disturbances such as movements of livestock by nomads and intensive animal breeding practices and/or natural disasters could create conditions conducive to enhanced transmission of flea-borne microbial and parasitic infections (Friggens and Beier 2010; Fakoorziba et al. 2011).

Pulex irritans can cause severe infestations in companion animals (Xhaxhiu et al. 2009), as well as man and his domesticated animals. It was observed more frequently than *C. canis* in 64 farms in Greece with a history of *P. irritans* infestations in their goats (Christodouloupoulos et al. 2006). This was also found to be true in the present study.

The largest level of flea infestation was observed in farms with intensive animal husbandry system. As stated above, animal shelter plays a significant role in the completion of life cycle of fleas, since it contains litter rich in organic matter which enables eggs to develop with many

hosts present upon emergence from cocooned pupae. Manure accumulation in animal shelters leads to increased warmth and humidity, which facilitates the multiplication of fleas and the richness of organic matter supplies food and protection for the developing larvae (Obasaju and Otesile 1980). In fact, eggs and larvae were found at a depth of up to 3 cm in the litter of intensive farms. This could also be exemplified in other insects' systems (Azizi et al. 2012).

In a recent study of flea infestation on farm animals of northwest Iran, three species of fleas were found on/off hosts with *P. irritans* representing 91.2 % of all identified fleas (Garedaghi 2011). In another similar study in north-west Iran, a clear majority (92.8 %) of fleas sampled from sheep, goat, hen, ox and man or from the floor of their dwellings belonged to *P. irritans* which substantiated the results in the present study (Rahbari et al. 2008). In addition, in both of these studies the most infested hosts were sheep and goat which was in line with the present findings. The human flea, *P. irritans*, was the most prevalent flea species in our study, since the samplings involved only hand catch method in a specific region during a limited interval of time.

Table 3 The frequency distribution of blood-fed male and female fleas, *Pulex irritans*, in relation to their anthropophagic preferences based on indirect ELISA test in different villages of Marvdasht County of Fars province, southern Iran

Village	Blood-fed Female (%)	Blood-fed Male (%)	ELISA-positive (%)	ELISA-negative (%)
Koohsabz	24 (3.20)	14 (1.87)	–	20 (10)
Ghasemabad-Ramjerd	21 (2.80)	10 (1.33)	–	–
Booreki	23 (3.07)	15 (2.00)	–	20 (10)
Shamsabad	23 (3.07)	27 (3.60)	–	20 (10)
Tajabad	20 (2.67)	9 (1.20)	2 (1)	18 (9)
Nasrabad	21 (2.80)	11 (1.47)	6 (3)	14 (7)
Fathabad	22 (2.93)	10 (1.33)	–	–
Firoozi	19 (2.53)	12 (1.60)	4 (2)	16 (8)
Jahanabad	20 (2.67)	24 (3.20)	–	20 (10)
Mahjenabad	20 (2.67)	17 (2.27)	–	–
Khaniman	13 (1.73)	35 (4.67)	–	–
Golijan	21 (2.80)	23 (3.07)	6 (3)	14 (7)
Maragolu	16 (2.13)	22 (2.93)	–	20 (10)
Dashtak	16 (2.13)	13 (1.73)	–	–
Barezabad	21 (2.80)	25 (3.33)	–	–
Hassanabad	15 (2.00)	17 (2.27)	–	–
Taleghani	17 (2.27)	16 (2.13)	12 (6)	8 (4)
Bizjanolia	18 (2.40)	15 (2.00)		
Baniyeke	17 (2.27)	25 (3.33)		
Sidan	26 (3.47)	17 (2.27)		
Total	393 (52.40)	357 (47.60)	30 (15)	170 (85)

The fact that most ELISA-positive fleas with the highest proportion (6 %) of human blood in their gut were from the Taleghani village with no small ruminant hosts indicated the potential importance of using specific zooprophylaxis in holding back infestation among people. On the other hand, the proximity of a few emaciated small ruminant hosts with high burden of fleas suggested not only that cohabitation of humans with animals is detrimental but also the health status of farm animals is of utmost significance in considering the infestation risks.

This was also the first study on fleas from animal to human dwellings in Marvdasht of Fars province, southern Iran. Further studies are required to determine the role of these fleas in pathogen transmission at each locality. It was concluded that *P. irritans* was widely distributed in this area. Based on haematophagic activity, human fleas posed a serious health threat to residents and their economically vital livestock.

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