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## Morphological and molecular characterization of *Ctenocephalides* spp isolated from dogs in north of Iran

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### Abstract

The main aim of the present study was to assess the infestation level of *Ctenocephalides* spp on domestic dogs in Meshkinshahr County, located in Ardabil province (north of Iran). A total of 20 domestic dogs were randomly selected for this study. After flea combing, results revealed that 100% of the dogs were infested with fleas. A total number of 942 fleas were collected. Two species were identified, of which *Ctenocephalides canis* the most abundant (98.73%) was followed by *C. felis* (1.27%). The dog flea, *C. canis* was the most common flea infesting 100% dogs and *C. felis* was identified on 7/20 (35%). The internal transcribed spacer 1 (ITS1) sequences of *C. canis* and *C. felis* collected from dogs to clarify the taxonomic status of these species. The results of PCR assay and sequence analysis did not show clear molecular differences between *C. canis* and *C. felis*.

**Keywords:** Flea, *Ctenocephalides canis*, *C. felis*, ITS1, Meshkinshahr, Iran

### 1. Introduction

Some ectoparasites such as fleas, louse, mites and tick of pet animals, can infest humans and other animals that may lead to the development of dermatitis and transmit vector-borne diseases [1]. Fleas are the most important ectoparasites of domestic animals. They are obligate haematophagous ectoparasites of mammals and birds. The fleas are small, laterally flattened and wingless. Sometime they can jump 2-3 meters. Fleas are of tremendous medical and economic importance as vectors of several diseases important to human health for example murine typhus, bubonic plague, flea-borne spotted fever and harboring *Bartonella* spp. Fleas may play a role as parasites causing allergic dermatitis or other conditions as a result of their feeding activities [2-4].

Animals such as dogs, may play an important role as bridging hosts for fleas of different wild animals, pet and humans. They will come into contact with different animals during their seeking behavior and therefore acquire the fleas of different animals. Dobler and Pfeffer have reviewed the published literature from 1980 to 2010 about the occurrence and frequency of fleas in the dog populations of different countries. They found that more than 15 different flea species have been described in domestic dogs, whereas the cat flea, *C. felis* was the most prevalent flea species found globally on dogs [5].

There are approximately 2,500 species of fleas. Within the family Pulicidae, the genus *Ctenocephalides*, includes 13 species and subspecies according to different morphological criteria based on the shape and structure of their genitalia and the presence and distribution of setae, spines, and ctenidia on the body [5, 6, 7]. Studies have found that *C. felis* and *C. canis* are the most common flea species living with dogs. *C. felis* and *C. canis* have been studied by different authors [4, 7-12]. In these studies, based on the shape of the head, the length of the first spine of the genal comb, number of spines in the met episternite, the distribution of spines in the hind tibia, and male and female genitalia, the *C. felis* and *C. canis* were characterized. Various studies have indicated that, fleas are most frequent canine ectoparasites reported in Iran [13, 14].

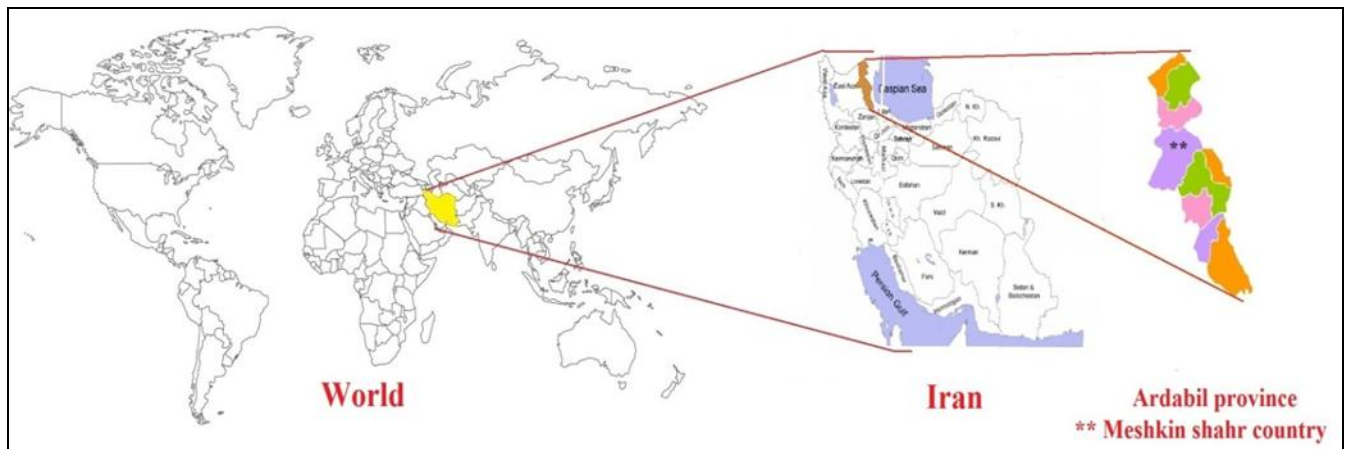
In the present work, a comparative morphological and molecular study was conducted on *C. felis* and *C. canis* that isolated from domestic dogs in Meshkinshahr County, located in Ardabil province, northwest of Iran.

## 2. Materials and Methods

Ardabil Province is located in the north of Iran, bordered by the Republic of Azerbaijan in the north, East Azerbaijan Province in East and Zanjan Province in South. The area of this province is 17953 square kilometers located 1830 meters above sea level. Ardabil city is its administrative center. The province is considered the coldest province in Iran. Large parts

of the province are green and forested. Its famous natural region is the Sabalan Mountains.

Meshkinshahr County is one of the counties located in Ardabil Province in the northwest part of Iran (Fig 1). Meshkinshahr County lies at 38° 44' N and 47° 40' E and is at an altitude of 4811 meters above sea level. At the 2006 census, the county's population was 156,000 in 36,000 families (Census 2006).



**Fig 1:** Location of Ardabil Province in which has been located in the North West part of Iran. Meshkinshahr County is one of the most important counties of the province.

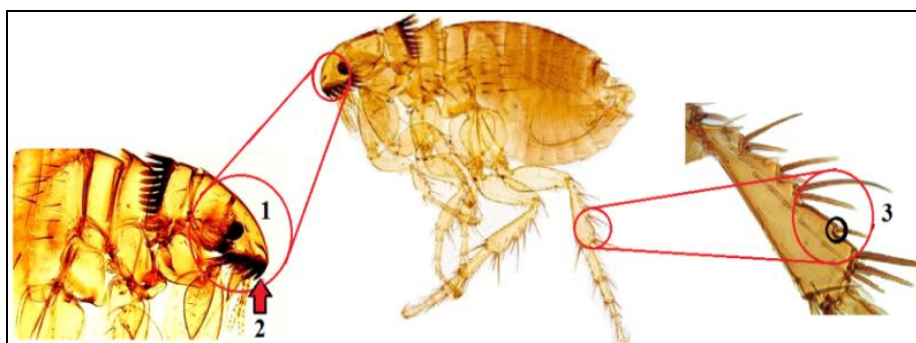
Dogs of all age groups and sexes in Meshkinshahr County were considered as study animals. Samples were collected at the time intervals between the months of August to October 2014. All fleas were collected using brushing against the hair of dogs. In some cases, the ectoparasites were collected by the use of forceps. Collected fleas were finally stored in 70% ethanol for their preservation and identification to the species level. Captured fleas were transported to laboratory located in Tarbiat Modares University (TMU), Tehran, Iran. In the laboratory, the fleas were cleaned with water and immersed in 10% potassium hydroxide (KOH) with a slightly warm for 1-3 hours [10]. Then, the fleas were transferred to 5% acid alcohol for 3 min to adjust the pH of samples. Dehydration of the samples was done by using series of alcohol from 50, 70, 80, 90, and 100%. Finally, the fleas were mounted on microscope slides. After mounting, all fleas were identified microscopically, according to the center of disease control (CDC) key [15].

To extract DNA from fleas, extraction methods of Ishorowicz was applied [16]. The fleas were crushed individually in sterile microtubes with the tip of a sterile pipette. Then, genomic DNA was extracted and preserved at -20 °C until use. The primer used by Marrugal *et al* was applied to identify the fleas [17]. These primers were specific to identify an ITS1 fragment of length (700 bp) of the ribosomal DNA (rDNA)

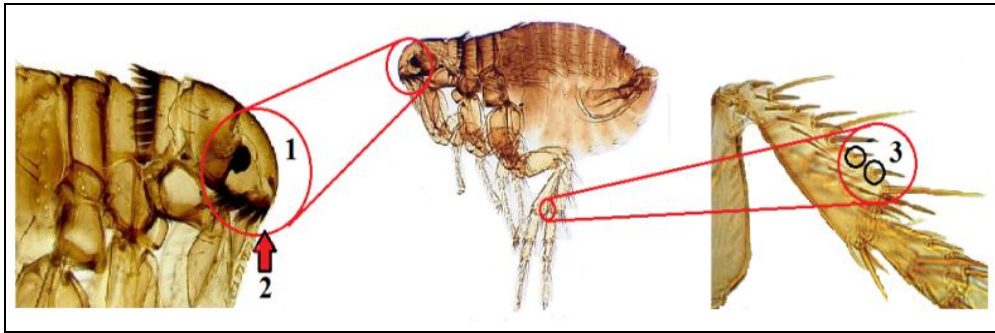
region. The forward primer was NC5 5'-GTAGGTGAACCTGCGGAAGGATCATT-3' and the reverse primer was ITS1rev 5'-GCTGCGTTCATCGACCC-3'. In each reaction a total of 15 µl of master mix 9µl, primers (F and R) 2 µl, DW 2µl, DNA 2 µl were added. The amplification conditions were: 94 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 30s; annealing at 58 °C for 30 s and extension at 72 °C for 40 s; with a final extension step at 72 °C for 10 min. The PCR product was visualized by UV transillumination in a 1.5% agarose gel after electrophoresis with Safe stain solution. Bands were eluted and purified from the agarose gel by using the Bioneer's DNA/RNA Extraction & Purification Kit. DNA was sequenced by Macrogen (Seoul, Korea, Tel.: +82-2-2113-7047, Email:info@macrogen.com).

## 3. Results

A total of 20 domestic dogs were randomly selected for this study. Of the 20 dogs, 35% (7/20) were female, while 65% (13/20) were male. A total of 942 fleas belonging to the genus *Ctenocephalides* were collected from dogs from different localities. Two species were identified, of which *C. canis* was the most abundant (98.73%) followed by *C. felis* (1.27%) (Fig 2 and 3). The dog flea, *C. canis* was the most common flea infesting 100% dogs and *C. felis* was identified on 7/20 (35%).



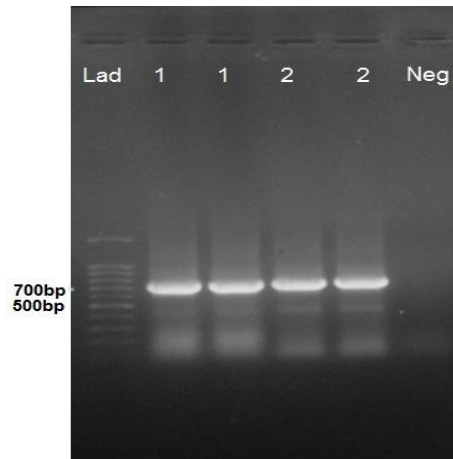
**Fig 2:** Adult of *C. felis*, 1; shape of the head, 2 length of the first spine of the genal comb and 3; One short, stout bristle in the interval between the postmedian and long apical bristles of the dorsal margin of the hind tibia.



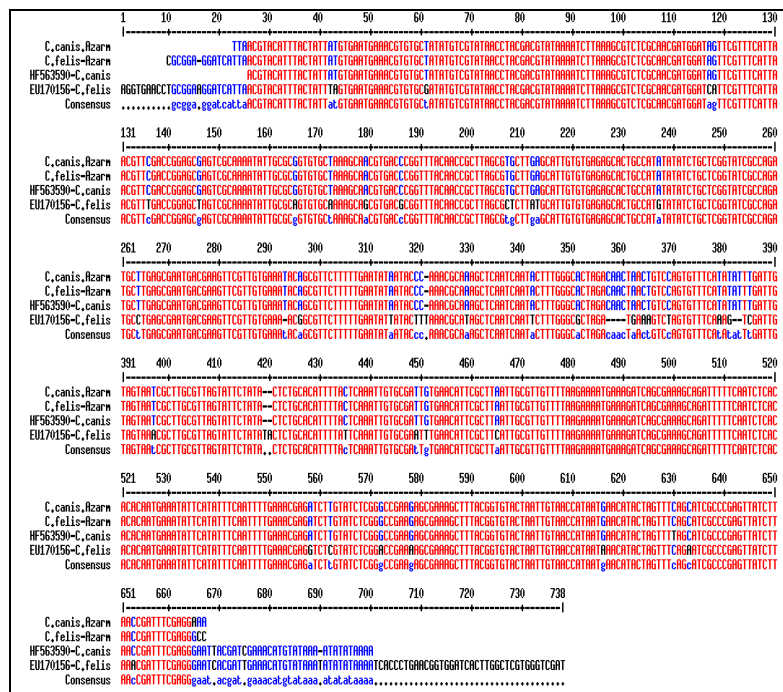
**Fig 3:** Adult of *C. canis*, 1; shape of the head, 2; length of the first spine of the genal comb, 3; Two short, stout bristles in the interval between the postmedian and long apical bristles of the dorsal margin of the hind tibia.

The PCR product was amplified from the genomic DNA of fleas isolated from dogs (Fig4). The ITS1 sequences of the rDNA of *C. felis* and *C. canis* were 700 bp in length (Fig5). When the ITS1 sequences of *C. felis* and *C. canis* were compared, it was found that these two sequences are likely to be 99 percent similar to each other. The results did not show clear molecular differences between *C. canis* and *C. felis*. The

comparative study of the ITS1 sequences of the different morphological populations observed in *C. canis* and *C. felis* did not show molecular differences. It can be concluded that, ITS1 region is not a good gene to approach different taxonomic and phylogenetic questions in *Ctenocephalides* species.



**Fig 4:** The PCR-based detection of fleas (Genus *Ctenocephalides*) DNA in dogs of Meshkin Shahr. The amplified 700-bp product from the positive sample for *C. canis* (lane 1) and positive sample for *C. felis* (lane 2) were subjected to electrophoresis in 1.5% agarose gel. A 100-bp ladder (Lad) and a negative control free of DNA template (Neg) were run in parallel.



**Fig 5:** Comparison and alignment of the consensus nucleotide sequences of the ITS1 for *C. felis* and *C. canis* isolated from on domestic dogs in Meshkinshahr country, located in Ardabil province, northwest of Iran.

#### 4. Discussion

The present result indicated that fleas are most common ectoparasite in domestic dogs of Meshkinshahr area. The fleas were studied morphologically and it was found that they belong to two species of *C. canis* and *C. felis*. *C. canis* had the highest infestation rate (100%) and a frequency (98.73%) and *C. felis* was found on 7/20 (35%) of dogs with a frequency of 1.27%. In the present study morphological characteristics of the fleas were in agreement with those cited by Gil Collado<sup>[18]</sup>, Beaucournu and Menier<sup>[11]</sup>, Beaucournu and Launay<sup>[8]</sup>, and Durden and Traub<sup>[12]</sup>. The greatest prevalence infestation of fleas was reported by Estareds *et al.* with 89% in two districts of San Juan de Lurigancho, San Martin de Porres, Comas and independence of Lima. Various studies have found that *C. canis* and *C. felis* are the two most common flea species of the dogs, however, the prevalence of these species varies in different geographic areas, for example *C. canis* being the most prevalent species in rural parts of the United Kingdom<sup>[20]</sup>, Greece<sup>[21]</sup>, Albania<sup>[22]</sup>, Turkey<sup>[23]</sup> and Australia<sup>[24]</sup> while *C. felis* was the dominant species in dogs in England<sup>[25]</sup>, Germany<sup>[26]</sup>, Spain<sup>[27]</sup> and Denmark<sup>[28]</sup>.

In a similar study by Aldemir on the prevalence of infection to ectoparasite in dogs in the Erzurum region in Turkey, from a total of 48 dogs, *C. felis* and *C. canis* were identified in 17 dogs (35.41%) which the most common ectoparasites were identified *C. canis*(31.25%) and *C. felis* (4.17%)<sup>[23]</sup>.

Several reports have documented ectoparasite infestations among dogs in Iran<sup>[13, 29-32]</sup>. The dog fleas and cat fleas have been reported on the dogs from different parts of Iran, but their prevalence was not noted<sup>[29]</sup>. In this study, results are similar to previous reports in different regions of Iran. For example, in the research that conducted by Jafari Shoorijeh and Bahrami *et al.* the most common ectoparasite on the dogs was *C. canis*. Jafari Shoorijeh *et al.* has recorded only 13.75% infestation by *C. canis* from dogs in Shiraz<sup>[30]</sup> where Bahrami *et al.* has recorded 28.89% and 2.44% infestation by *C. canis* and *C. felis* from dogs in Iran and Iraq border line district<sup>[13]</sup>.

Scarce studies have been carried out on molecular differentiation of canine fleas. In our study, the ITS1 region of the rDNA of *C. felis* and *C. canis* were used to differentiate within species of fleas. A similar study has been conducted by Vobis *et al.* to carried out a molecular phylogeny of isolates of *C. felis* based on analysis of the internal transcribed spacers 1 and 2 (ITS1 and ITS2)<sup>[33]</sup>. Comparing *C. felis* and *C. canis* sequences, indicated that these two sequences are likely to be 99 percent similar to each other. So, our result did not show clear molecular differences between *C. canis* and *C. felis*. This fact is not in agreement with Marrugal *et al.* who found different lengths in the ITS1 rDNA among *C. canis* and *C. felis* isolates from different geographical regions<sup>[17]</sup>.

#### 5. Conclusion

It is concluded that ITS1 region is not an appropriate gene for identifying two species of *Ctenocephalides* of dog.

**5.1 Conflict of Interest:** The authors declare that they have no conflict of interest.

**5.2 Statement of Animal Rights:** All procedures performed in studies involving animals were in accordance with the ethical standards of the Tarbiat Modares University Ethical Committee.

#### 6. Acknowledgements

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