



Research Article

Flea Species Isolated from the Human and Animals: Prevalence and Ultrastructural Studies

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ABSTRACT

Fleas are one of the insects that belong to the order *Siphonaptera*. They are a wingless, compressed laterally, and non-host specific insect parasitizing a wide range of hosts, so they are able to transmit diseases from animals to human. This study aimed to identify the fleas in and around human and animals with their prevalence and ultrastructure study. Flea samples were collected from four governorates (Cairo; Giza; Fayoum and South Sinai); Egypt during the period from January 2017 to May 2018. One hundred and twenty fleas were collected from five animals (donkeys, sheep, goats, dogs, and cats) surrounding humans. All the collected fleas were identified as *Ctenocephalides felis felis* (*C. f. felis*). All the animals were infested with *C. f. felis* with flea's allergic dermatitis recorded in dogs, human, and donkeys, while goats and sheep showed anemic mucous membrane. The highest infestation was recorded in spring and summer. The morphological characters of 50 specimens were recorded and measured using the stereoscopic and light microscope as well as scanning electron microscope (SEM). The ultramorphological characters of *C. f. felis* head, thorax, and abdomen with the genital organs of male and female were fully described. The antennae have three segments with the third one having several adhesive circular disks and numerous sensory hairs in antennal grooves. The maxillary palps were well developed with four segments which supported with sensory small hairs. The first genal comb is half of the second one or nearly equal to its length. These results about identification and prevalence studies were used to update the knowledge about the flea species present in investigated localities in Egypt; to detect the appropriate control measures which could be applied in flea's infestation.

Key words: *Ctenocephalides felis felis*, *Flea allergic dermatitis*, *Siphonaptera*, Prevalence, Ultrastructure

INTRODUCTION

Fleas are blood-sucking ectoparasites of human, animals, and birds. They are non-host specific wingless insects, parasitizing a wide range of hosts. So, they can transmit diseases from animals to humans and between animals (Kettle, 1995).

Ctenocephalides felis felis (*C. f. felis*), the cat flea is a non-host-specific type of fleas that has a wide range of hosts all over the world. It acts as an intermediate host for *Dipylidium caninum*, some filarial nematodes, and different pathogens as *Rickettsia felis* and *Bartonella* spp. (Kettle, 1995 and Šlapeta *et al.*, 2018). It infests a wide range of mammals including sheep, goats, cattle, buffaloes, horses, and donkeys which reared in the barn with straw bedding in which the life cycle can develop well. Death was reported in young unusual hosts (lamb and calves) which were severely infested with *C. f. felis* (Yeruham *et al.*, 1989).

The cat flea is the most dominant flea type in domesticated and even wild animals in different countries such as China, where all the fleas collected were *C. f. felis* (Šlapeta *et al.*, 2011). In comparison, in Australia, *C. f. felis* is the predominant flea on animals in veterinary clinics in which no *Ctenocephalides canis* cases were recorded (Šlapeta *et al.*, 2011 and Lawrence *et al.*, 2015). The life cycle of *C. f. felis* is defined as complete metamorphosis. It lasts for about 14 days at 32°C and may be as long as 140 days at low temperature, at 13°C and humidity above 50%. All stages of fleas are present on dusty corners, feces and housing bedding (Kettle, 1995 and Soulsby, 1986). The geographical distribution of the fleas is dependent upon the presence of its hosts; while *C. f. felis* has multiple hosts so, it also has a worldwide distribution. In Egypt; a recent arthropod survey on donkeys which reveals 58.33% infestation with *C. f. felis* by Attia *et al.* 2018. So, the aim of the current studies is to update the knowledge about the

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Fleas present in different localities in Egypt in different animals which surround the human with special references to their ultramorphological structure.

MATERIALS AND METHODS

Collection of fleas

The fleas samples were collected from four governorates (Cairo, Giza, Fayoum, and South Sinai) during the period from January 2017 to May 2018; the Cairo governorate is located at E $^{\circ}07'14031$ and N $^{\circ}30'0230$, and the other governorates are far from Cairo by 7, 70 and 244 km, respectively. The fleas were collected from different animals, such as donkeys; goats; sheep; dogs; cats, animals which come in contact with human (Tables 1). The fleas which were collected from human as (human keeping the sheep herd and human rearing a pet animals) the number of human 30 collected in spring and autumn in the highest percentage of infestation in all animals. All Institutional and National Guidelines for the care and use of animals were followed.

Preservation of fleas

The collected fleas were divided into two tubes; one tube had 70% ethanol and the other tube had 2.5% glutaraldehyde [diluted in PBS (Phosphate- Buffered Saline; pH: 7.4)]. Then all samples were sent to the parasitology department, Faculty of Veterinary Medicine, Cairo University, Egypt for further studies.

Identification of the collected fleas

The identification of the collected fleas was carried out according to the morphological characters reported by (Kettle, 1995 and Soulsby, 1986). The morphological characters from 50 specimens were measured under the light microscope (Labomed $\times 40$ and $\times 100$).

Preparation of permanent specimens

For detailed morphological structures, all fleas were fixed immediately in 70% ethanol to stop their movement. After the fleas' relaxation, they were mounted according to (Attia, 2018). Briefly; all the fleas were placed in 5% sodium hydroxide (NaOH) for 1 hour then washed with distilled water and dehydrated in ethanol series for 1 hour each. The specimens were cleared in clove oil then put in Xylene. Finally, all specimens were mounted using Canada balsam and incubated at 40°C overnight in order to dry (Attia and Salaeh, 2019).

Preparation of specimens for scanning electron microscope (SEM)

The ultrastructure of the fleas was identified using SEM, according to (Attia and Salaeh, 2019). Summarized as, the fleas were washed using buffered saline (PH 7.2) then fixed in 2.5% glutaraldehyde at 4°C for 24 hours as described by (Hilali *et al.*, 2015). All fleas were dehydrated in ascending ethanol series (10% -30%-50% -70% -90% and 100%) and desiccated on CO₂ critical point drier (Autosamdri-815, Germany). Flea specimens were glued and coated on gold; photographed with SEM (JSM 5200, Electron prob, Microanalyzer, Jeol, Japan) at Faculty of Agriculture, Cairo University, Egypt. Finally, fleas were identified according to (Beaucournu and Ménier, 1998).

RESULTS

The collected fleas

One-hundred and twenty fleas were collected from the four governorates. The fleas were identified as *C. f. felis*. The fleas were collected from different animals (donkeys; goats; sheep; dogs; cats) around the humans. All the examined animals were infested with *C. f. felis* with different infestation degrees which were the highest in spring and summer (the highest percentage occurring on goats) in comparison to autumn and winter. All of the animals had different clinical manifestation as, fleas allergic dermatitis recorded in dogs, human and donkeys, anemic mucous membrane recorded in goats. Prevalence studies of *C. f. felis* on different hosts which come in contact to humans recorded in Table 2.

All the fleas which were collected from human were identified as *C. felis felis* in spring and summer (100%).

Adult fleas

Under stereoscopic loupe; fleas were dark brown and wingless insects with a laterally compressed body of about 1-3.5 mm mean length. The whole length of female body ranged from $2-3.5\text{ mm} \pm 0.3$ and whole length in male $1-2.5\text{ mm} \pm 0.5$. They possess six long and strong legs. Ultramorphological structure of *C.f. felis*.

The body composed of head; 3 segmented thorax and large abdomen. The head has an acutely angled frons which specific for *C. f.felis* and pointed clypeus with the head length $350-500\text{ }\mu\text{m} \pm 0.6$. (Fig. 1). The antennae have three segments with the third one being well developed with several adhesive circular disks with its length $140-180\text{ }\mu\text{m} \pm 0.7$ and numerous sensory hairs in antennal grooves which are present around the edge of the groove (Fig. 2). The maxillary palps were well developed with four segments which supported with sensory small hairs at their posterior end (Fig. 3), with the presence of two maxillae at the posterior end of the head. There were two simple eyes at the two sides called ocelli, the genal combs which are present posteriorly to the ocelli; were eight in numbers and present on each side of the head; the first genal comb is half of the length in comparison to the second one ranging from $60-80\text{ }\mu\text{m}$ which is important criteria to distinguish this fleas with other Siphonaptera (Fig. 1 and 3). The number of postocciptal hairs were from 5-10 in number. The thorax is composed of three segments; the first thoracic segment (prothorax) is supported with eight pronotal combs; with total dimension of prothorax were $100-150\text{ }\mu\text{m} \pm 1.3$, and the pronotal length was $140-180\text{ }\mu\text{m} \pm 1.1$. The lateral metanotal area (LMA) has two lateral setae (Fig. 4 and 5). The legs are supported with two long claws which are radiated in shape and two triangular pullvilli. The mesothorax dimension was $150-200\text{ }\mu\text{m} \pm 2$; and in metathorax $150-300\text{ }\mu\text{m} \pm 1.6$. The posterior end of the abdomen has several sensory sensillum which contains several sensory pits with sensory hairs which are opposite with the antisensilial seta and posterior to it and there can be seen several stylets called anal stylet. The posterior end of the male has a clasper with numbers of bristles on its outer surface which is supported by manubrium in abdomen and at the surface there is an apical arm of 9th sternite.

Table 1: collection of fleas in different governorates in Egypt.

Locality	Number of the collected fleas	Number of the collected male and female fleas
Cairo	20	12♀ and 8♂ <i>C. f. felis</i>
Giza	30	25♀ and 5♂ <i>C. f. felis</i>
Fayoum	50	42♀ and 8♂ <i>C. f. felis</i>
South Sinai	20	15♀ and 5♂ <i>C. f. felis</i>
Total number of collected fleas	120	

Table 2: Prevalence studies of *C. f. felis* on different hosts which come in contact to humans.

Animals Seasons	Donkeys			Sheep			Goats			Dogs			Cats		
	No. Ex.	No. Pos.	%	No. Ex.	No. Pos.	%	No. Ex.	No. Pos.	%	No. Ex.	No. Pos.	%	No. Ex.	No. Pos.	%
Spring	36	20	55.5	30	20	66.6	30	25	83.33	20	15	75	25	20	80
Summer	36	30	83.3	25	20	80	30	30	100	25	18	72	30	25	83.3
Autumn	36	15	41.6	25	8	32	30	15	50	30	15	50	30	20	66.6
Winter	36	5	13.8	25	5	20	30	8	26.6	30	6	20	30	10	33.3
Total	144	70	48.6	105	53	50.5	120	78	65	105	54	51.4	115	75	65.2

No. Ex.: number of examined animals; No. Pos.: number of positive animals for *C. f. felis*; %: percentage of infestation.

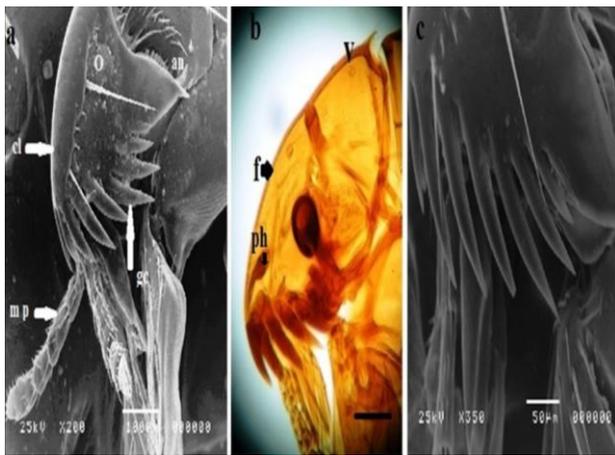


Fig. 1: Scanning electron micrograph (SEM) of *C. f. felis* head; A: showing genal comb (gc) with the first spine equal to the second one; maxillary palp (mp) and simple eye (Ocelli) (o) and clypeus (cl) with antenna (an); b: permanent mount of cat flea head showing pharynx (ph) frons (f); vertex (V) and also the genal comb well developed and the maxillary palp; C: large magnification of SEM showing the shape of genal comb.

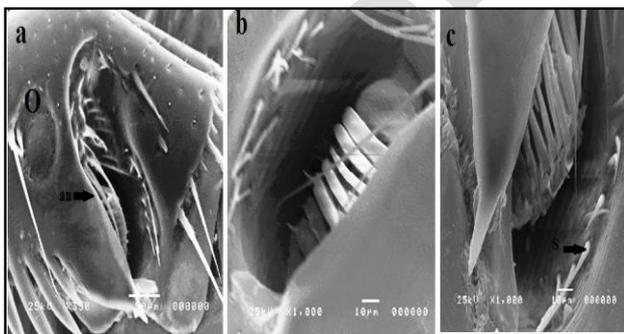


Fig. 2: Scanning electron micrograph (SEM) of *C. f. felis* head; a; b; c: showing antenna (an) which has 3 segments, the third one being well developed with a number of adhesive circular discs and many sensory hairs (s) in antennal grooves which were present around the edge of the groove; O: ocelli.

The abdomen of female with its posterior end showing several sensory sensillum and posterior to it, a number of stylets called anal stylets; and had female genital organ which is the spermatheca; tall and head. (Fig. 6 and 7).

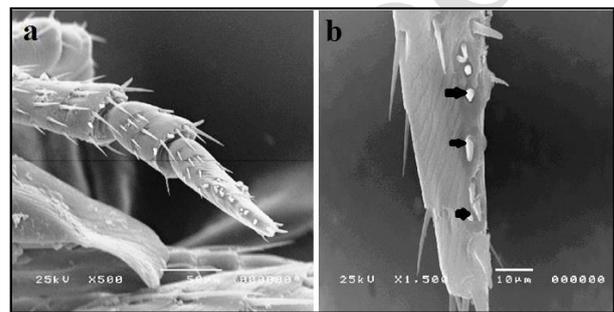


Fig. 3: Scanning electron micrograph (SEM) of *C. f. felis* head showing in a and b; the maxillary palps having 4 segments which are supported with sensory small hairs at their posterior end (showed by arrows).

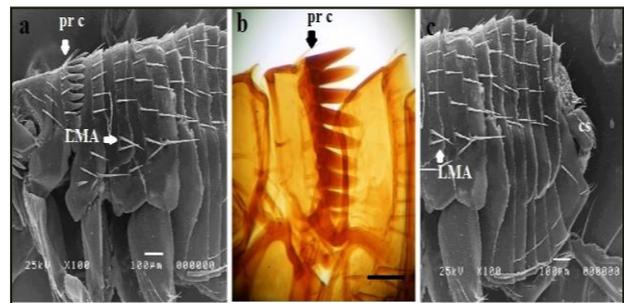


Fig. 4: Scanning electron micrograph (SEM) of *C. f. felis* thorax; a: The thorax composed from three segments; the first thoracic segment (prothorax) supported with 8 pronotal combs (pr c). The metanotal area (LMA) of *C. f. felis* bearing 2 setae. b: light microscopic photograph of *C. f. felis* thorax showing 8 pronotal combs (pr c). c: abdomen of male *C. f. felis* showing; the lateral metanotal area (LMA) which bears two setae and claspers at the posterior end (cs); in photo (b): Scale bar: 100µm.

DISCUSSION

The cat flea (*C. felis felis*) is world-widely distributed and it has a wide range of hosts because it lacks the host specificity (Yeruham and Koren, 2003) and is important to livestock as well as pet animals (Rust, 2017). Several flea important transmitted diseases were explained with Blagburn and Dryden (2009) with fleas allergic dermatitis and many diseases transmitted. In this study, with severe infestation of the examined animals and human with the world-wide species of the fleas, we mention that it is

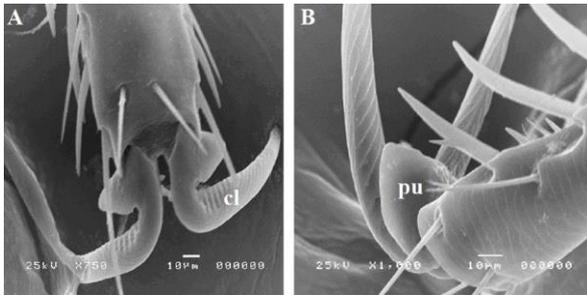


Fig. 5: Scanning electron micrograph (SEM) of *C. f. felis* legs: a;b showing the legs which are supported with two long claws (cl) which are radiated and also have two triangular Pullvilli (pu).

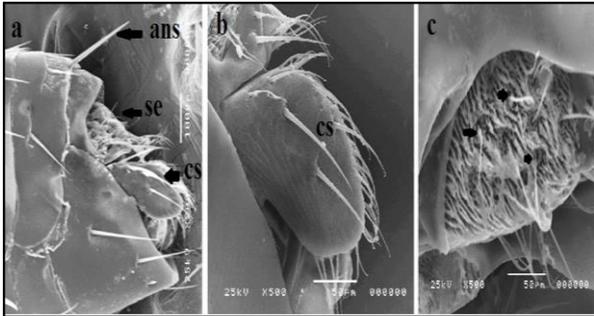


Fig. 6: Scanning electron micrograph (SEM) of male *C. f. felis* abdomen: a; b: The posterior end has several sensory sensillum (se) which contain several sensory pits with sensory hairs (pointed by arrows in (c)); the sensillum is opposite with the antisensilial seta (ans). The posterior end of the male has a clasper (cs) with numerous bristles on its outer surface in a; b.

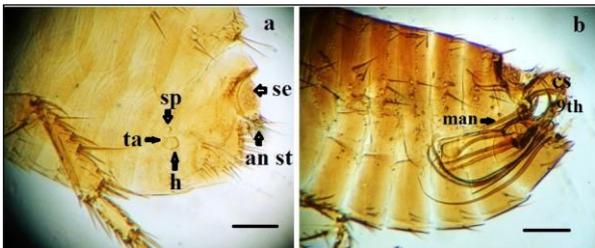


Fig. 7: Light microscopic photograph of *C. f. felis* abdomen: a: abdomen of female with its posterior end showing several sensory sensillum (se) and posterior to it, a number of stylets called anal stylets (an st); the sp: spermatheca; ta: tall; h: head. The posterior end of the male has a clasper (cs) which is supported by manubrium in abdomen and at the surface there is an apical arm of 9 th sternite. Scale bar: 100 μ m.

important to find a possible way to control such disease infestations. Studies on successful control strategies of fleas were based on the identification of the parasites, their life cycle, and bionomics of their different stages. The life cycle of *C. f. felis* and *C. canis* had been studied several times (Baker and Elharam, 1992). The adult *C. f. felis* begins feeding within a few minutes from emergence. Fleas mate after the blood meal and the female starts the egg-laying (Dryden and Rust, 1994). Eggs are laid on the host nest, bedding wool, and carpet (Dryden and Rust, 1994). The hatched *C. f. felis* larvae are free-living, they feed on organic matter and the adult flea feces. The larvae become a cocoon in which they pupate (Shanks *et al.*, 2000). The explanation of the life cycle was to determine the exact time for fleas' control.

Different survey studies were done in only one or two animals species as in Israel by Yeruham and Koren; 2003 on donkeys and by Attia *et al.* 2018 on donkeys in Egypt. Survey occurred in water buffalo in India by Singh *et al.* 2011. Different morphological studies were done on *C. f. felis* but none gave the full description of the different parts of fleas with scanning electron microscope as (Kettle, 1995, Lawrence *et al.*, 2015 and Marrugal *et al.*, 2013). Recent study on morphological and molecular studies on *Ctenocephalides* spp by Azarm *et al.* 2016, this study occurred on one animal species which is the dogs while our study occurred in different animals species and human. The previous study gives light microscopic explanation of the two species *C. canis* and *C. felis* which based on description of the shape of the head and legs setae (Azarm *et al.* 2016). So, our results give the exact morphological identification on the widely spread fleas in Egypt, *C. f. felis*.

Conclusions

The high prevalence and infestation of *C. felis felis* in different domestic animals could be due to the direct contact of cats with goats, sheep, and donkeys as well as the presence of dogs which collect these fleas from these animals. These animals surround humans so the fleas infest the human of the same species as animals which are *C. felis felis*. There must urgently be found the way to control these fleas by using different insecticides, a topic which had been severely studied before.

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