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Genotyping of the cat flea (*Ctenocephalides felis*) parasitizing domestic small ruminants in Erbil – the Kurdistan region of Iraq

Abstract

Small ruminants such as sheep and goats are deemed an important economic source in the Kurdistan region. Infestations with haematophagous ectoparasites such as fleas, which are common vectors in transmitting serious bacterial diseases, pose a great menace on the public health and animal welfare. To meet the requirements of the study, convenient sampling and molecular characterization were used to investigate infestations of sheep and goats with the cat flea, *Ctenocephalides* (*C.*) *felis* in Erbil governorate. A total of 400 samples (200 of sheep and 200 of goats) from 12 ruminant farms at five districts from intensive and semi-intensive breeding systems were examined. The overall prevalence of the cat flea infestation was 65.79% and 34.21% for goat and sheep respectively. High infestation by cat flea (80.32 ± 2.377) was observed in ruminant reared in intensive management system as compared to those of the semi-intensive management system (4.760 ± 0.8109). Molecular characterization of mitochondrial cytochrome oxidase I gene and sequencing results confirmed the morphological identifications. The accession numbers: MZ156870, MZ156871, MZ156872, MZ156873 and MZ156874 for *C. felis* from Sheep and goats were allotted in NCBI. To the best of our knowledge, this is the first molecular study of *C. felis* in small ruminants in the Kurdistan region and Iraq. Since cat fleas can infest various hosts, this could pose a threat of pathogen transmission, therefore further studies are required.

Keywords

Genotyping, cat, flea, *Ctenocephalides felis*, ruminants, domestic

RESEARCH ARTICLE

Genotyping of the cat flea (*Ctenocephalides felis*) parasitizing domestic small ruminants in Erbil – the Kurdistan region of Iraq

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ABSTRACT

Small ruminants such as sheep and goats are deemed an important economic source in the Kurdistan region. Infestations with haematophagous ectoparasites such as fleas, which are common vectors in transmitting serious bacterial diseases, pose a great menace on the public health and animal welfare. To meet the requirements of the study, convenient sampling and molecular characterization were used to investigate infestations of sheep and goats with the cat flea, *Ctenocephalides (C.) felis* in Erbil governorate. A total of 400 samples (200 of sheep and 200 of goats) from 12 ruminant farms at five districts from intensive and semi-intensive breeding systems were examined. The overall prevalence of the cat flea infestation was 65.79% and 34.21% for goat and sheep respectively. High infestation by cat flea (80.32 ± 2.377) was observed in ruminant reared in intensive management system as compared to those of the semi-intensive management system (4.760 ± 0.8109). Molecular characterization of mitochondrial cytochrome oxidase I gene and sequencing results confirmed the morphological identifications. The accession numbers: MZ156870, MZ156871, MZ156872, MZ156873 and MZ156874 for *C. felis* from Sheep and goats were allotted in NCBI. To the best of our knowledge, this is the first molecular study of *C. felis* in small ruminants in the Kurdistan region and Iraq. Since cat fleas can infest various hosts, this could pose a threat of pathogen transmission, therefore further studies are required.

Keywords: Genotyping, cat, flea, *Ctenocephalides felis*, ruminants, domestic

1. INTRODUCTION

Small ruminants are important income sources for farmers in the Kurdistan region of Iraq. It is estimated that Iraq has 18.615 million sheep and 1.897 million goats including Kurdistan. This has made sheep and goats play a great influence on the agro-economic status of people through their products such as meat, milk, wool, hair and hides. Infestations of these animals with ectoparasites cause great economic loss through deteriorating animal's health, growth rate and production performance (Ajith et al., 2017). Among the significant ectoparasites are fleas, which are obligate blood feeding parasites of mammals and birds (Durden and Hinkle, 2019, Lewis, 1993). Fleas are well known as vectors of pathogens such as rickettsia typhi, murine typhus, plague and feline leukemia as well as intermediate host for filarids

and cestodes. Additionally, they cause irritation and restlessness, flea allergy dermatitis, iron deficiency anemia and even death of the host during heavy infestation (Fular et al., 2020, Lawrence et al., 2014, Shakya et al.).

Over 2,500 species and subspecies of fleas (Order: Siphonaptera) exist worldwide. Among most of the medically important fleas, are those in the genus *Ctenocephalides* (Durden et al., 2005). *Ctenocephalides felis*, commonly "cat's flea" represents two recognized subspecies: *Ctenocephalides felis strongylus* (Jordan, 1925) and *Ctenocephalides felis felis* (Bouche, 1835)(Singh et al., 2011). In addition to infesting cats and dogs, *C. felis felis* has been reported to parasitize many species of wild and domestic animals worldwide particularly in the tropical and

sub-tropical regions (Araujo et al., 1998, Kaal et al., 2006). The morphological identification of *Ctenocephalides* species is not confirmative and not reliable due to the close similarities of the key identification body parts of the flea (Jacob and Sengupta). Therefore, molecular characterization is more decisive for the identification and classification of

2. MATERIALS AND METHODS

2.1. Study Area

Fieldwork was conducted in 5 districts of Erbil, Iraq, the region is found 65 km northeast of Erbil with an altitude ranging from 1250-1350 meters above sea level. The topography of the zoon is mostly marked by the presence of a number of mountains, plateaus, hilly, and slope areas. The average amount of rainfall, temperature, and relative humidity are, 980mm, 24C, and 23.9% to 79% respectively. Sampling was conducted from April to September 2020. Two farms were randomly selected from each of the five districts according to their farm management systems, Intensive and semi-intensive. Farms were identified and randomly selected from the database of the Department of Veterinary and animal resource, Erbil.

2.2 Sample size:

The minimum number of animals used for this study was 400 (200 of sheep and 200 of goats) to measure the prevalence of *C.felis felis*. The sample size of ectoparasite was determined based on the predictable prevalence of 50% and the absolute desired precision of 5% at 95% dependability level. The desired sample size was calculated using the standard formula described by (Thrusfield, 2018).

2.3. Methodology

Parameters such as age, gender, and farm management practices were recorded prior to the physical examination and sample collection. The selected sheep and goats were physically examined for the presence of fleas. The fleas were collected manually (by forceps) and preserved in separate vials containing 70% ethanol (Urquhart et al., 1996). Fleas were identified based on the morphology and morphometry (Jacob and Sengupta, Tylor et al., 2007).

Adult fleas, larvae and eggs were also searched in dung using floatation method, in which 0.5 kg of dung along with some soil and vegetation, were gently stirred in a large plastic container about two-third filled with water. Fleas and debris, such as crusts of dung pads and pieces of vegetation, floated to the surface and were skimmed off by forceps or with a fine sieve (Houston et al., 1982).

2.4. Fleas extraction and amplification

Genomic DNA was isolated from five adult individuals, each specimen was extracted by ZYMO Quick-DNA Tissue/Insect Microprep Kit manufactured USA- No. D6015. Polymerase Chain Reaction (PCR) was used to identify the fleas. A set of primers were used to amplify 700 bp partial sequence of mitochondrial COI in 50 µl of reaction mixture (table 1).

The primers were: forward C1-J-1718

(5-GGAGGATTTGGAAATTGATTAGTTCC-3) and reverse C1-J-1718

the parasite even at the subspecies level. Mitochondrial cytochrome oxidase I gene (COI) plays a pivotal role in classification because its variation allows species level discrimination (Deagle et al., 2014). Hence, the aforementioned gene was use in this study to characterize *C. felis felis* infestation in sheep and goats.

(5TAAACTTCAGGGTGACCAAAAAATCA-3).

Table 1: COI PCR Amplification Reagents

No.	PCR components	Concentration	Volume (µl)
1	Master Mix	2x	25
2	Forward Primer	20 Pmol	3
3	Reverse Primer	20 Pmol	3
4	DNase free Water	-	15
5	Template DNA	50ng/µl	4
Total			50

PCR was performed using (PTC-200 Gradient thermocycler) starting with step one as an initial denaturation at 95 C for 5 min, followed by 35 cycles of a denaturation at 95C for 35 second , annealing at 58C for 35 sec. , an extension at 72C for 1 min and then folloed with final step of extra extension at 72C for 10 min. Amplified sampled were elecrophorized in 1.5% agarose gel for 30 minutes and then visualized under UV trans-illuminator. Five positive samples were sequenced by ABI Prism Terminator Sequencing Kit (Applied Biosystem) at Macrogen Center in Korea. Chromatograms of COI gene were edited and base calls checked using Finch TV program software. The COI gene sequences were applied to publically available Alignment Search Tool, BLAST (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) and compared to other biological sequence to find out similarities with other targets.

3. Results

3.1 Fleas prevalence in relation to barn systems

The highest mean of flea infestation was seen in farms practicing an intensive management system (80.32 ± 2.377) whereas in semi- intensive management system was 4.760 ± 0.8109 , and there was high significant difference between the two systems according to number of fleas (Figure 1).

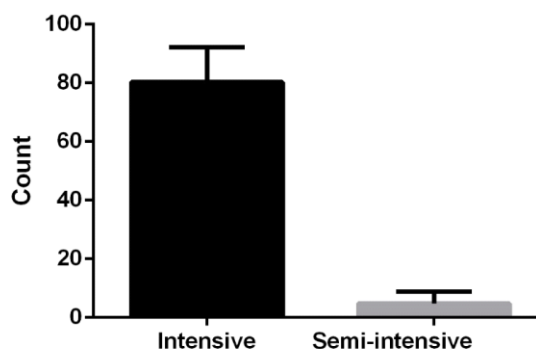


Figure (1): Total count of fleas collected in dung according to rearing systems of ruminants.

The load of fleas collected from dung from different sites varied with the highest mean of 93 at 85.81-100.2 (95% CI), and lowest mean of 65.6 at 53.83-77.37 (95% CI) (Table 2).

Table 2: Comparison of the overall number of fleas in dung according to collection site

Locations	N	Mean	SD	SEM	95% CI
loc1	5	74.2	6.419	2.871	66.23-82.17
loc2	5	65.6	9.476	4.238	53.83-77.37
loc3	5	81.2	8.556	3.826	70.58-91.82
loc4	5	87.6	4.879	2.182	81.54-93.66
loc5	5	93	5.788	2.588	85.81-100.2

Out of these 12 farms, heavy flea infestation was noticed on goats (which were reared on intensive managements with dung on floor) than sheep in same system management whereas the goats which were reared on the semi-intensive managements were with light flea infestation. Fleas showed more affinity for goats than sheep (Figure 2).

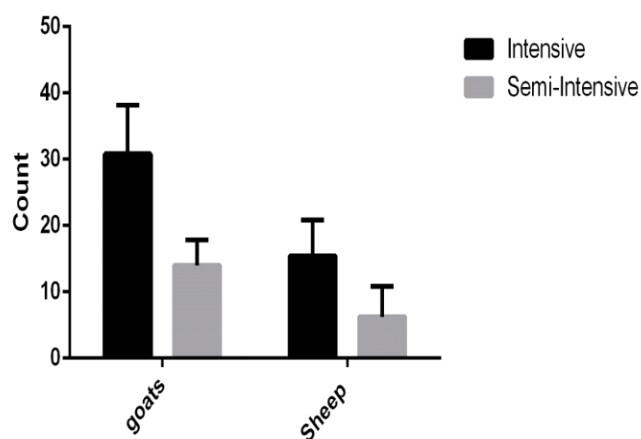


Figure2: Prevalence of fleas among goats and sheep in relation to the system of rearing.

3.2 PCR amplification and sequencing

The PCR amplifications of mitochondrial COI partial gene using forward C1-J-1718 and reverse C1-J-1718 primers yielded a replication product of nearly 700 bp (Figure 3). The sequencing results of the present study indicated 100% homology to *C. felis felis* investigated from different hosts in different geographical areas. Sequences were submitted to NCBI and allotted accession numbers, MZ156870, MZ156871, MZ156872, MZ156873 and MZ156874.

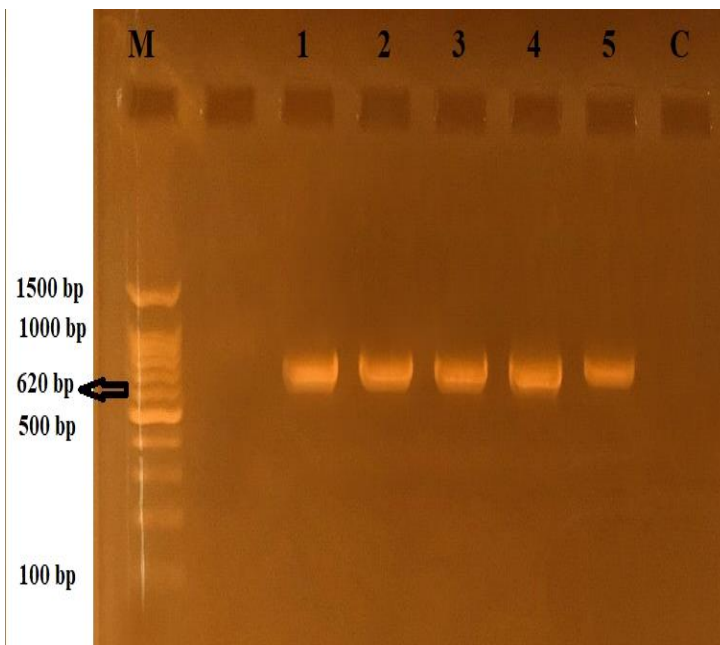


Fig. 1: PCR amplification of 700 bp partial Cytochrome C oxidase I gene from five *C. felis felis* amplicants.

4. Discussion

In the present study, sheep and goats were investigated for the purpose of determining the prevalence of the cat flea *C.felis felis* based on both morphological and molecular methods. Ruminants in intensive breeding system showed more likely to be infested with the parasites compared to the semi-intensive system. This is in agreement with other studies (Kaal et al., 2006, Soundararajan et al., 2018) Barns with accumulation of dung plays an important role in the development of fleas since accumulation of litter material on the floor favors the development of eggs, hatching of larvae and pupae and proliferation of fleas (Obasaju and Otesile, 1980). It is concluded that the dung on the floor is the main source for breeding of fleas (Soundararajan et al., 2018). The condition allows manure to accumulate in animal houses results in increased warmth and humidity, with favors the proliferation of fleas (Dipeolu and Ayoade, 1982) and the abundance of organic matter provides nutrition and protraction for the developing larvae. This was confirmed by the finding of eggs and larvae at a depth of up to 3 cm in the litter of intensive farms (Kaal et al., 2006).

The abundance of fleas varied according to the collection sites ranging from the highest mean of 93 at 85.81-100.2 (95% CI) to the lowest mean of 65.6 at 53.83-77.37 (95% CI). Traditional free-range rural animal production system forms an integral part of farming systems. The provision of the simple shed with low-cost housing materials and the differences among farmers caring methods are the most factors affecting flea infestation. The occurrence and spread of these problems correlated with host factors, poor management, climatic factors, feed scarcity and

inadequate veterinary services (Dipeolu and Ayoade, 1982).

Regarding host type, higher infestations of fleas were observed in goats compared to sheep in both intensive and semi-intensive systems. Accumulation of litter material on the floor favors the development of eggs, hatching of larvae and pupae and proliferation of fleas (Obasaju and Otesile, 1980, Soundararajan et al., 2018). Agro-climatic region, breed, immune status, system of rearing and hygiene were highly affecting the prevalence and distribution of ectoparasites among goats (Ajith et al., 2017). The prevalence of ectoparasites depends on several environmental, host and parasite related factors like herd density, hygiene, climate, system of rearing, age, sex, host immune status, parasite biology etc. (Tylor et al., 2007). Goats are constantly attacked by fleas especially *C. felis* (Fagbemi, 1982, Joseph, 1981, Kaal et al., 2006, Yeruham et al., 1989).

5. Conclusion

This high prevalence suggests that these fleas are very common, and present major problems with regard to the health, and performances of these important animals in the study area. It is concluded that the dung on the floor is the main source for breeding of fleas. The farmers and farm owners were advised to remove the dung daily. Traditional free-range rural animal production system which forms an integral part of farming systems, in almost all rural communities in Erbil is characterized by mismanagement, malnutrition, theft, predation, diseases and parasites infestation resulting in high mortality and low productivity. The molecular method (PCR) is a confirmative and a method of choice for detection of flea species infesting ruminants. Other studies of the parasite particularly on feline are required to better understand the source of infestations.

Conflict of interest: No conflict of interest

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