SHORT COMMUNICATION



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Survey of flea infestation in cats and dogs in Western Andalusia, Spain: Seasonality and other risk factors for flea infestation

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Abstract

This epidemiological survey aims to provide an update on the main flea species that parasitize domestic animals in the Western Andalusia assessing several ecological features that could be considered as possible risk factors for flea infestation. Over a 19-month period (June 2021 to January 2023), we obtained a total of 802 flea samples from 182 dogs (Canis lupus familiaris, Carnivora: Canidae, Linnaeus, 1758) and 78 cats (Felis silvestris catus, Carnivora: Felidae, Schreber, 1775). For each parasitized host, an epidemiological survey was completed, including the following information: geographical origin, age, sex, rural or urban habitat, type of animal's lifestyle (domestic or non-domestic), health status, cohabiting or not with other animals and the total number of collected fleas. The most common species was Ctenocephalides felis (Siphonaptera: Pulicidae) (Bouché, 1835) with a total of 713 specimens, which accounted for 89% of the total fleas. The second most abundant species was Pulex irritans (Siphonaptera: Pulicidae) (Linnaeus, 1758) with a total of 46 collected fleas (6% of the total). The remaining species identified were Archaeopsylla erinacei (Siphonaptera: Pulicidae) (Bouché, 1835) (25 specimens), Spilopsyllus cuniculi (Siphonaptera: Pulicidae) (Dale, 1878) (12 specimens) and Ctenocephalides canis (Siphonaptera: Pulicidae) (Curtis, 1826) (6 specimens), which accounted for 3%, 1% and 1%, respectively, of the total fleas collected. The months with the highest number of collected fleas were, in ascending order, May 2022, September 2021 and July 2021. Dogs had a greater diversity of flea species, and flea sex ratios were female biased in all identified species and among all studied hosts. Finally, we identified some potential host risk factors that promoted higher flea intensities, such as living in rural areas, or presenting other pathologies.

KEYWORDS

Ctenocephalides felis, epidemiology, fleas, Pulex irritans, Spain, Spilopsyllus cuniculi

INTRODUCTION

The Order Siphonaptera includes secondarily wingless holometabolous insects with a worldwide distribution. This group is comprised of about 2700 species of hematophagous parasites described so far (Hastriter et al., 2018). Fleas have historically had significant

epidemiological importance, not only directly through their bites but also because of their ability to act as arthropod vectors for a multitude of pathogenic bacteria that cause several infectious diseases (Eisen & Gage, 2012). It is worth highlighting the importance of the influence of climate change on the epidemiology and distribution of vector arthropods such as fleas. Not only does temperature influence the

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vectors, but the pathogens they transmit are also conditioned by temperature for their multiplication in such a way that it affects not only them but also their relationship with viruses, bacteria and parasites (Caminade et al., 2019). Within this context, the region of Andalusia, located in the southern part of Spain, has been a key location for analysing and studying the potential expansion of certain vector arthropods. Its proximity to the African continent, combined with its unique climatic characteristics, make this region an ideal epidemiological model site for studying the possible establishment of vectors and the infectious diseases they transmit in areas where they had not been previously recorded (Escoz Roldán, 2022).

The latest fleas recorded from mammals from Andalusia included several species such as Ctenocephalides felis, C. canis, Pulex irritans, Archaeopsylla erinacei, Spilopsyllus cuniculi and Nosopsyllus barbarus (Siphonaptera: Ceratophyllidae) (Jordan and Rothschild, 1912) (Zurita et al., 2021). Furthermore, many of the flea species mentioned above have been widely cited for their potential vectorial role in the transmission of numerous pathogenic bacteria such as several Rickettsia (Da Rocha Lima, 1916) and Bartonella (Strong et al., 1915) species (Bitam et al., 2010).

The main objective of this study was to provide an epidemiological update on the main flea species that parasitize domestic animals in the Western Andalusia. The study focused on analysing which flea species were most prevalent on host animals that have a closer relationship with humans, specifically dogs and cats. Furthermore, updated epidemiological data were provided on the most prevalent species of fleas, along with the ecological characteristics of their hosts, to help determine the most appropriate control and prevention measures against these vectors.

MATERIALS AND METHODS

Over a 19-month period, we obtained flea samples from dogs and cats. To collect fleas from all these hosts, we contacted various veterinary clinics, veterinary hospitals, pet shelters and some pet owners. To conduct our study, we contacted a total of 145 veterinary clinics and 30 pet shelters and kennels. Out of these, 18 centres agreed to collaborate in the collection of samples (see Acknowledgements). They all participated in this sampling voluntarily. Only animals parasitized by fleas were sampled. Veterinary practitioners performed an initial inspection of dogs and cats brought to their practices. Each pet was inspected for fleas and examined by a veterinarian who recorded clinical signs related to flea infestation. Adult flea counts on dogs and cats were conducted as described in the World Association for the Advancement of Veterinary Parasitology guidelines (Marchiondo et al., 2013). Briefly, dogs and cats were combed over the entire body with a fine-toothed comb for 5–10 min.

All veterinary centres and individuals that participated in our study were in the Western Andalusia, specifically in different towns in the provinces of Seville, Cádiz, Córdoba and Huelva (Table 1 and Figure 1). Within the province of Seville, fleas were collected from a total of 30 different localities; whereas, in Córdoba, Huelva and Cádiz,

the number of localities included in this study was 5, 14 and 2, respectively (Table 1). For each parasitized host, an epidemiological survey was completed, including the following information: Geographical origin, age, sex, rural or urban habitat, type of animal's lifestyle (domestic or non-domestic), health status, cohabiting or not with other animals and the total number of collected fleas.

The sample collection period encompassed late June 2021 to January 2023. All captured fleas from each infested host were transferred to a small plastic 1.5 mL tube containing 96% ethanol until processing. Then in our lab, fleas were sexed and identified to species using a CX21 microscope (Olympus, Tokyo, Japan). Diagnostic morphological characters of all the samples were studied by comparison with figures, keys and descriptions reported by Beaucournu and Launay (1990).

Data were collected in a datasheet (Microsoft® Excel® for Microsoft 365 MSO version 2306). The burden of fleas is reported as mean \pm standard deviation. Chi-square test, or Fisher's exact test where appropriate, were used whereby the number of parasitized individuals was compared between habitat types (urban vs. rural), lifestyle (domestic vs. non-domestic), health condition (healthy vs. sick/other parasites) and the presence of other animals (cohabiting vs. isolated), with p < 0.05 regarded as statistically significant. All statistical analyses were performed with R commander (Rcmdr), the graphical user interface for R software, version 4.3–1. Flea intensity was calculated according to the method of Yin et al. (2011): flea intensity = total number of fleas/number of infested hosts (dogs/cats).

RESULTS

Species of fleas and hosts

In the present study, a total of 802 fleas belonging to five different species were collected from dogs and cats: C. felis, C. canis, A. erinacei, P. irritans and S. cuniculi (Table 1). Out of the total collected fleas, 519 (65%) were obtained from dogs and 283 (35%) from cats (Table 1). Furthermore, the most common species was C. felis with a total of 713 collected specimens, which accounted for 89% of the total fleas. The second most abundant species was P. irritans, with a total of 46 collected fleas (6% of the total). The remaining species identified were A. erinacei with 25 collected specimens, 12 fleas classified as S. cuniculi and 6 fleas as C. canis, which accounted for 3%, 1% and 1%, respectively, of the total fleas collected (Table 1). Of all the fleas collected from cats, 96% of them (n = 271) were identified as C. felis, whereas the remaining fleas were S. cuniculi. Conversely, from the fleas collected from dogs, a total of four species were identified (C. felis, C. canis, P. irritans and A. erinacei), with C. felis being the predominant species, representing 85% of all fleas isolated from dogs (Table 1).

Regarding the number of parasitized host animals, samples were obtained from a total of 260 different hosts. Of these, 70% (n=182) were dogs, whereas 30% (n=78) were cats. Based on the total number of fleas collected and the total number of infested hosts, the total flea intensity observed in dogs in this study was 2.9, whereas for cats, this value was 3.6.

 TABLE 1
 Species, geographical origin, gender and hosts of the total flea samples collected in this study.

Species	Locality/province	Number of fleas (female/male)	Hosts (N)
Archaeopsylla erinacei	Dos Hermanas, Sevilla	14 (8/6)	Hedgehog (1)
	Dos Hermanas, Sevilla	23 (15/8)	Dogs (3)
	Fuente Palmeras, Córdoba	2 (1/1)	
		Total: 39 (24/15)	
Pulex irritans	Cazalla de la sierra, Sevilla	5 (5/0)	Dogs (21)
	Alcalá del Río, Sevilla	8 (5/3)	
	Cantillana, Sevilla	4 (3/1)	
	Arahal, Sevilla	1 (0/1)	
	Aguadulce, Sevilla	2 (2/0)	
	Estepa, Sevilla	1 (1/0)	
	Herrera, Sevilla	1 (0/1)	
	Pilas, Sevilla	1 (1/0)	
	Jabugo, Huelva	2 (2/0)	
	Galaroza, Huelva	6 (4/2)	
	Ayamonte, Huelva	1 (0/1)	
	Fuente Palmeras, Córdoba	14 (7/7)	
		Total: 46 (30/16)	
pilopsyllus cuniculi	Fuente Palmeras, Córdoba	12 (7/5)	Cat (1)
		Total: 12 (7/5)	
tenocephalides canis	Cazalla de la sierra, Sevilla	3 (2/1)	Dogs (4)
	El Repilado, Huelva	2 (1/1)	
	Galaroza, Huelva	1 (1/0)	
		Total: 6 (4/2)	
Ctenocephalides felis	Sevilla, Dos Hermanas	8 (4/4)	Cats (77)
	Sevilla, Sevilla	85 (65/20)	
	Tomares, Sevilla	25 (20/5)	
	Valencina de la Concepción, Sevilla	7 (6/1)	
	Aznalcázar, Sevilla	1 (1/0)	
	Alcalá de Guadaira, Sevilla	6 (5/1)	
	Brenes, Sevilla	1 (1/0)	
	Espartinas, Sevilla	1 (1/0)	
	Écija, Sevilla	13 (11/2)	
	Lantejuela, Sevilla	7 (6/1)	
	El Viso del Alcor, Sevilla	3 (3/0)	
	Tocina, Sevilla	1 (1/0)	
	Los Rosales, Sevilla	1 (0/1)	
	La Puebla del Río, Sevilla	41 (34/7)	
	Montequinto, Sevilla	2 (2/0)	
	El Puerto de Santa María, Cádiz	10 (7/3)	
	Galaroza, Huelva	2 (1/1)	
	Huelva, Huelva	1 (1/0)	
	Minas de Río Tinto, Huelva	1 (1/0)	
	Nerva, Huelva	2 (1/1)	
	Ayamonte, Huelva	12 (12/0)	
	Fuente Palmeras, Córdoba	11 (8/3)	

Species	Locality/province	Number of fleas (female/male)	Hosts (N)
	Posadas, Córdoba	30 (26/4)	
		Total: 271 (217/54)	
	Sevilla, Sevilla	5 (3/2)	Dogs (162)
	Tomares, Sevilla	1 (1/0)	
	San Juan de Aznalfarache, Sevilla	1 (1/0)	
	Palomares, Sevilla	29 (19/10)	
	Cazalla de la sierra, Sevilla	30 (21/9)	
	El Pedroso, Sevilla	4 (2/2)	
	Constantina, Sevilla	6 (3/3)	
	Alcalá del Río, Sevilla	1 (1/0)	
	El Viso del Alcor, Sevilla	8 (6/2)	
	Pilas, Sevilla	42 (36/6)	
	Carrión de los Céspedes, Sevilla	1 (1/0)	
	Huévar del Aljarafe, Sevilla	1 (1/0)	
	Villamanrique de la Condesa, Sevilla	1 (1/0)	
	Arahal, Sevilla	4 (0/4)	
	Écija, Sevilla	20 (16/4)	
	Aguadulce, Sevilla	1 (0/1)	
	Marinaleda, Sevilla	1 (1/0)	
	Estepa, Sevilla	12 (10/2)	
	San Fernando, Cádiz	2 (2/0)	
	El Puerto de Santa María, Cádiz	55 (42/13)	
	Galaroza, Huelva	5 (4/1)	
	Jabugo, Huelva	1 (1/0)	
	Fuenteheridos, Huelva	2 (2/0)	
	Alájar, Huelva	18 (9/9)	
	Santa Ana la Real, Huelva	2 (0/2)	
	Higuera de la Sierra, Huelva	6 (5/1)	
	Cortegana, Huelva	8 (4/4)	
	Campofrío, Huelva	1 (1/0)	
	Nerva, Huelva	5 (2/3)	
	Minas de Río Tinto, Huelva	3 (3/0)	
	Ayamonte, Huelva	21 (18/3)	
	Sanlúcar de Guadiana, Huelva	1 (1/0)	
	Fuente Palmeras, Córdoba	85 (68/17)	
	Posadas, Córdoba	44 (31/13)	
	Almodóvar del Río, Córdoba	9 (7/2)	
	Rivero de Posadas, Córdoba	4 (3/1)	
	La Carlota, Córdoba	2 (2/0)	
		Total: 442 (328/114)	

Period of sampling

As mentioned above, the sampling period was from late June 2021 to January 2023. From the end of June 2021 to the end of that same year, a total of 461 fleas were collected corresponding to 56% of the total fleas collected. The remaining samples were obtained throughout the year 2022 until the end of January 2023. If we observed the total number of fleas obtained month by month, we could observe that during 2021, the months with the highest number of collected samples were July, August, September and October, whereas, in 2022,

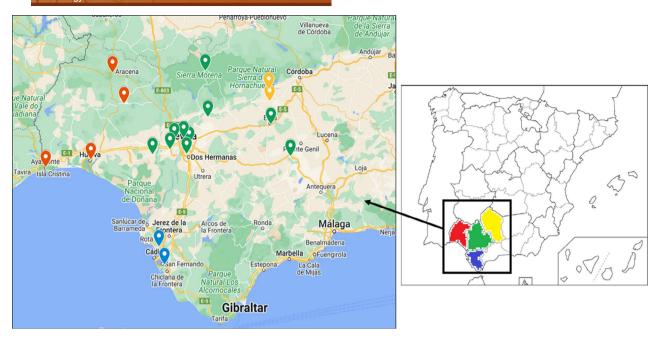


FIGURE 1 Overview of the sampling locations in the four Andalusian provinces: Cádiz (blue spots), Córdoba (yellow spots), Huelva (red spots), Sevilla (green spots). Map edited using the application 'MyMaps-Google Maps (https://www.google.es/maps)', 'Map data ©2023 Google'.

the months with the highest number of samples obtained were May, June and July. Lastly, out of all the months during the sampling period, the top 3 months with the highest number of collected fleas were, in ascending order: May 2022, September 2021 and July 2021.

Flea sex ratios

Out of the total of 802 fleas obtained, 75% (n = 602) were females, whereas 25% (n = 200) were identified as males (Table 1). If we analyse the differences by sex within each identified species, we can observe that in all five species present in this study, the percentage of females is greater than that of males. By species, the largest difference in the number of males and females collected was observed in C. felis, with a 76% of all studied specimens being females (n = 545).

Flea-host ecological features

Almost 44% (n=352, 191 ± 137.2) of the fleas were collected from young animals (under 2 years old), whereas almost 48% (n=382; 176 ± 22.6) came from older animals (more than 2 years old). Out of the total fleas collected from parasitized cats, 56% (n=160) were obtained from kittens or young cats (flea intensity = 3.1), whereas 33% (n=94) were collected from adult cats (flea intensity = 5.5). In the case of dogs, the percentages were reversed, with 55% of the fleas (n=288) obtained from dogs older than 2 years (flea intensity = 3) and 37% from puppies or young dogs under 2 years old (flea intensity = 2.8). For a total of 68 fleas, data about host age were not provided.

When analysing the host habitats, 60% (n=492; 191 ± 137.2) of the total collected fleas originated from hosts living in rural areas, whereas the remaining 40% (n=310; 176 ± 22.6) were from hosts residing in urban habitats. The proportion, approximately 60%–40%, between both habitats remained consistent both in the number of analysed hosts and in the number of collected fleas from both dogs and cats. In terms of infestation intensity, we recorded values of 3.2 for dogs and 3.9 for cats from rural habitats, whereas these values were lower for dogs and cats living in urban habitats (2.5 and 3.2, respectively).

If we analyse the lifestyle of the hosts, we observed that almost 60% of the parasitized dogs were domesticated; conversely, in the case of the cats, most of them (over 80%) were non-domesticated cats. In this sense, we observed that, in total, nearly 60% (n=481; 160.3 ± 127.2) of the flea samples were from non-domesticated hosts without daily contact with humans, whereas the remaining 41% (n=321; 111.7 ± 160.8) came from domesticated hosts. Accordingly, flea intensities were higher on non-domesticated dogs and cats (3.2 and 3.9, respectively) compared with domesticated ones (2.6 for dogs and cats).

Lastly, we examined the health status of the infested hosts and the presence or absence of other animals cohabiting with them. About 22% ($n=181,\,182.3\pm151$) of the collected fleas originated from isolated hosts, whereas 67% ($n=547;\,60.3\pm68.6$) came from hosts cohabiting with other animals. Conversely, 66% ($n=534,\,267\pm103.2$) of the fleas were collected from healthy hosts, whereas almost 24% ($n=189;\,94.5\pm89.8$) were from sick hosts.

Regarding dogs, approximately 75% of them exhibited a healthy status, devoid of any apparent injuries, diseases or other ectoparasite infestations. Furthermore, nearly 55% of these dogs lived alongside

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other animals, with the most common cohabiting species being horses, sheep, other cats and dogs, hedgehogs and even chickens. When discussing fleas collected from dogs, almost 65% were obtained from healthy dogs, further, 60% were collected from dogs cohabiting with other animals, showing flea intensity values of 2.5 and 3, respectively. These values corresponded with 4 for non-healthy dogs and 2.3 for dogs living isolated from other animals.

In the case of cats, these percentages and values appeared slightly different. Approximately 75% of them showed an optimal state of health, without the presence of other ectoparasites; however, more than 80% of them lived alongside other animals, particularly other cats, dogs and rodents. These percentages aligned with the fleas obtained based on these ecological characteristics. Hence, nearly 70% of the fleas collected from cats came from cats in good health, and 80% of them were obtained from cats cohabiting with other animals in their habitat. Flea intensity was practically the same for both healthy cats and those that presented some form of apparent disease or showed infestations by other ectoparasites (3.2 and 3.1, respectively). Alternatively, flea intensity was lower in those cats cohabiting with other animals (3.7) compared with those cats living isolated from other animals (4.2).

We detected statistically significant differences between parasitdomestic versus non-domestic animals $p = 3.999e^{-10}$) and isolated versus cohabiting with other animals $(\chi^2 = 31.11, p = 0.000689)$. Contrasts in flea parasitation between animal health condition (healthy vs. sick) ($\chi^2 = 2.15$, p = 0.1428) and habitat types (rural vs. urban) ($\chi^2 = 1.18$, p = 0.2775) did not differ significantly.

DISCUSSION

Most flea epidemiological studies published so far have been focused on fleas collected from dogs and cats, although numerous publications also evaluated the presence of these ectoparasites in peridomestic animals such as hedgehogs. The most common species of flea collected from the European hedgehog is A. erinacei (Zurita et al., 2021). Despite the host specificity of this flea, it is noteworthy that in our study, this species was also found on dogs cohabiting with nearby hedgehogs. This fact demonstrates the ability of this species to expand its range of hosts, thereby increasing its epidemiological significance. Additionally, our study represents the first evidence of the presence of A. erinacei parasitizing dogs in Spain.

As previously mentioned, five different flea species have been identified in our study (C. felis, C. canis, A. erinacei, P. irritans and S. cuniculi). This work represents the first evidence of S. cuniculi parasitizing cats and P. irritans parasitizing dogs in the province of Córdoba, Spain. In agreement with previous studies (Gálvez et al., 2016), C. felis was the most prevalent flea, constituting 87% of the total fleas obtained from both dogs and cats; however, dogs exhibited a greater diversity of flea species. Despite the observed disparity in flea diversity between dogs and cats, further research is necessary to comprehensively understand the underlying factors

contributing to the variation in flea species distribution between these two different hosts before drawing formal conclusions.

Our results once again demonstrate that C. felis is established as the dominant flea species in dogs and cats worldwide due to its remarkable ability to adapt to numerous hosts and its broad tolerance of a range of environmental conditions (Lawrence et al., 2019). However, P. irritans has emerged as the second most prevalent species on dogs, which is a novel finding compared with the previous studies conducted in Spain where C. canis appeared second to C. felis in terms of prevalence (Gálvez et al., 2016; Gracia et al., 2012). In addition, the presence of S. cuniculi, a species specific to rabbits (Oryctolagus cuniculus. Lagomorpha: Leporidae. Linnaeus. 1758), on cats should be taken into consideration, as there have been studies exploring the potential implication of S. cuniculi in the transmission of certain diseases, such as myxomatosis caused by the myxoma virus (Mead-Briggs & Vaughan, 1975).

The number of female fleas collected was consistently higher in all identified species and among all studied hosts. This differential pattern between males and females has been consistently evident in almost all epidemiological studies on fleas published so far (e.g., Gracia et al., 2012). Female-biased sex ratios in fleas may also be influenced by the more active males (looking for females) which may be more prone to becoming detached from the host or being subjected to greater host grooming activities (Krasnov, 2008). The difference in infestation intensity between dogs and cats was another notable finding in our study. We observed a higher infestation intensity in cats compared with dogs in agreement with Beck et al. (2006). This variation emphasises the complex dynamics of flea infestations and the importance of considering multiple factors that may influence infestation patterns in different host species and environments.

Our study confirmed that the period with the highest flea infestation corresponds to late spring, throughout the summer and early autumn, with a peak of flea number commonly observed in July. These findings are consistent with other similar studies conducted both in Spain (Gálvez et al., 2016) and in different regions of the northern hemisphere with diverse climatic conditions (Beck et al., 2006). All these data support the preference of the predominant flea species in pets for seasons with higher temperatures and lower percentages of rainfall.

With respect to host age, we observed contrasting patterns when comparing fleas collected from dogs and cats based on the age of the hosts. In this sense, Liberman et al. (2011) concluded that a host of a particular age could not be unequivocally predicted to be beneficial for fleas than a younger or an older host. However, Farrell et al. (2023) argued that possible reasons are wide ranging and speculative and include greater attraction of fleas towards younger animals because their increased exposure to outdoor environments exploring their surroundings and interacting with other animals may attract more fleas. Nevertheless, the higher total number of fleas found in adult hosts might be due to their longer exposure to potential flea habitats and their larger body size, which could support a higher flea burden.

Analysing our results based on lifestyle of infested hosts revealed a clear epidemiological pattern. Both total number of fleas and the intensity of infestation were higher in non-domesticated hosts (without close contact with humans) and rural habitats. These findings underscore the importance of both ecological characteristics as significant risk factors for potential flea infestations in specific hosts. Hosts living in rural habitats, such as feral or stray cats and dogs, are likely to have more frequent contact with wildlife, which can serve as reservoirs for fleas and other ectoparasites. In addition, rural areas with a higher flea intensity of wildlife and domestic animals may create a suitable environment for flea proliferation. Our findings are in broad agreement with numerous epidemiological studies showing a consistent pattern of higher infestation intensity in non-domesticated hosts and rural habitats (Farkas et al., 2009). However, although most epidemiological studies mentioned that increased contact with other animals is considered an important risk factor for possible flea infestation (Beck et al., 2006; Farkas et al., 2009), based on our results, we could not demonstrate this epidemiological pattern, and we were unable to draw a well-founded conclusion at this point.

Lastly, if we analyse the data based on the health status of the studied hosts, flea intensity values, especially in dogs, appeared much higher in those with some additional health problems constituting a possible risk factor for a potential flea infestation. In these cases, as some authors have argued (Schmid-Hempel, 2011), a decrease in host immune system defences could be directly related to higher parasitation rates. However, it is important to note that relatively little is known in this area since the relationship between the immune system and flea infestation is complex and may vary depending on the specific circumstances of each individual host and flea species involved (Krasnov, 2008).

This work represents the first epidemiological study on flea infestation in cats and dogs conducted in Western Andalusia, Spain. Based on our results, we demonstrated that C. felis is the dominant flea species on dogs and cats in this area, although dogs exhibited a greater diversity of flea species, and the number of female fleas was higher than males in all identified species and among all studied hosts. Alternatively, differences observed in the total number of flea collected in different months are significant as they provide valuable insights into the ecological dynamics of fleas and their host populations. Understanding these patterns is crucial for implementing effective flea control measures and preventive strategies during the periods of increased flea activity. In addition, considering environmental factors might help to identify additional risk factors for flea infestations in domestic hosts. All these epidemiological data can help to develop effective flea control and prevention strategies and improve the overall well-being of pets and other animals in different environments.

AUTHOR CONTRIBUTIONS

Cristina Cutillas: Conceptualization; validation; formal analysis; resources; writing - review and editing; visualization; supervision; funding acquisition; project administration. Antonio Zurita: Conceptualization; software; validation; formal analysis; investigation; data

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflicts of interest to report regarding the present study.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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