Research Article



Retrospective Seasonal Parasitological Survey on Prevalence and Epidemiological Determinants of Ectoparasitic Infestations in Dogs and Cats of Damietta, Egypt

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Abstract | Animal owners should be well-trained to deal with the potential that arises from the lack of all the purposive biosecurity measures planned to meet all the demands of dogs and cats. The study aimed to conduct a seasonal crosssectional survey on the point prevalence (PP) of ectoparasitic infestations in dogs and cats concerning some host, agent, and environmental determinants. A cross-sectional study was designed to last for four successive seasons from September 21st, 2020 to September 20th, 2021. A total of 1393 cats and 1511 dogs were admitted and examined for parasitic infestations. $PP_{infestations}$ revealed highly significant (P < 0.01) increases during fall and spring and fall in dogs and cats respectively; at < 1-year dogs and cats during all seasons; and during winter in males and spring in female dogs, summer in males, and winter in female cats. $PP_{infestations}$ revealed highly significant (P < 0.01) increases in German dogs and Persian cats in the four seasons; during fall in black and Tan dogs and spring in white-coated cats; and during summer in the single and fall in the multiple housing system of dogs and during summer in the single and spring in the multiple housing system of cats. Highly significant (P < 0.01) increases during spring, spring, winter, winter, and spring seasons in dogs and spring, spring and summer, spring, and fall seasons in cats consumed dry, cooked, raw, canned, and mixed food respectively. Species-specific $PP_{infestations}$ revealed highly significant (P < 0.01) increases in fleas during spring, ticks during summer, skin mites, and lice during fall in dogs, fleas during spring, and ear mites during the fall season in cats. Parasitological examinations identified Rhipicephalus sanguineus ticks, Ctenocephalides canis flea, Heterodoxus spiniger lice, and Sarcoptic scabiei mites in dogs, Otodectes cynotis ear mites, and Ctenocephalides felis flea in cats. Prevalence of parasitic infestations in dogs and cats showed strong associations with the breed, sex, age, coat color, housing system and pattern, type of food, and type of infesting external parasites concerning seasonal variations.

Keywords | Cross-sectional, Dogs, External parasites, Prevalence, Seasonal

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INTRODUCTION

The human-animal bond has been strengthened with dogs and cats with their role as companions to relieve depression, lower stressful conditions, and provides powerful health benefits. On the other hand, this close contact with dogs and cats might contribute to an increased risk of contracting some infectious and zoonotic

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diseases (Abu-Madi et al., 2016). Biosecurity measures should include standards and management factors that are set to meet all the physiological demands of dogs and cats regarding health status monitoring and reporting, proper design houses, routine examination, proper hygienic measures, feed and water of good quality, strategies of waste disposal, cleaning and disinfection procedures, medication and vaccination needs, and proper handling (De Leeuw, 2003). Animal owners should be aware and well-trained to deal with the potential that arises from the lack of all the purposive biosecurity measures planned (Macpherson, 2005).

Ectoparasites including ticks, lice, and mites are organisms that parasitize the host's skin for serving their livability, multiplication, maturation, and metamorphosis. Parasites are two-winged (dipterous) flies and their larvae usually invade the living and/or necrotic tissue of both animals and humans (Mansour et al., 2017). Parasitic infestation happens to occur with the availability of good microclimatic conditions like increased temperature and decreased relative humidity, so the summer and springtime represent the most dangerous time for parasitic attacks in companion fur animals such as dogs and cats (Shoorijeh et al., 2008). Parasitic infestations contribute to cost-effective production as they cause cardiovascular disorders including congestive heart failure and anemia (Zendehfili et al., 2015). They also formulate wounds that can act as a gate for the entrance of secondary bacterial and viral agents, as well they inject their toxins into the bloodstream of their host contributing to intoxication (Tong et al., 2019).

External parasitism enhabits a mean for the mechanical transmission of enteric microorganisms such as E. coli or Salmonella species and pyogenic micro-organisms such as Corynebacteria, Staphylococcus, or Streptococcus species (Kwak et al., 2021; Apanaskevich and Apanaskevich, 2016). They also enhabits a mean for the biological transmission of blood protozoa such as Babesia (red water), Thileria, and Trypanosoma species; bacterial microorganisms such as Pasteurella pestis; and some viral pathogens such as yellow fever virus (Apanaskevich et al., 2019; Shirazi et al., 2013). Prevalence (point and period) as an epidemiological measure is known for determining a specific parameter in a given duration of time (Bruce et al., 2017). Prevalence calculation usually depends on a randomly selected target population to increase the representation of the measured parameters (Kenneth, 2012). The measured prevalence is usually affected by a variety of factors that might be included in the study concerning host factors such as age, sex, breed, coat color, body size, and configurations, agent factors such as survivability, pathogenicity, infectivity, specificity of infection, and host range, and environmental

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factors such as temperature, humidity, airflow, rainfall, and seasonal variations (Kruse and Schuz, 2016).

The current study was designed to conduct a seasonal cross-sectional annual survey for four consecutive seasons on the most prevalent ectoparasites that parasitize the external surface of dogs and cats with concern to animal determinants such as age, sex, breed, coat color, agent determinant such as pathogenicity and specificity, and environmental determinants such as food and housing system and pattern.

MATERIALS AND METHODS

ETHICAL APPROVAL

The study design and animal management system were approved by the Scientific Research Ethics Committee on Animal and Poultry researches, Faculty of Veterinary Medicine, Suez Canal University, Egypt with approval number (2021029).

STUDY PERIOD AND LOCATION

The study was conducted for four successive seasons (Fall, winter, spring, and summer) from September 21st, 2020 to September 20th, 2021. The study was carried out in Damietta governorate, Egypt.

Damietta governorate as revealed in Figure 1 is located in the northeastern part of Egypt with coordinates of 31.3626° N in Latitude and 31.6739° E in longitude. Damietta has a surface area of 1.029 km² or about 5% of the Delta's area (Figure 1). Damietta has a desert climate. There is virtually no rainfall during the year. This climate is considered to be BWh according to the Köppen-Geiger climate classification.

STUDY DESIGN

A cross-sectional study was designed to last for a year of four successive seasons (Fall, winter, spring, and summer) from September 21st, 2020 to September 20th, 2021. During the study period, the dog and cat cases that were admitted to a veterinary clinic in Damietta governorate were recorded and inspected for ectoparasitic infestation. Data collection was carried out with more concern for some factors including animal species, breed, sex, age, coat color, housing system and pattern, type of food, and type of infesting external parasites if any. Special attention was given to the vaccination, medication, previous surgeries, fluid therapy, and deworming history of the dog and cat cases admitted.

During the study period, a total number of 1511 dogs and 1393 cats were admitted to the veterinary clinic and examined for external parasite infestations. The ambient

macroclimatic temperature and relative humidity were recorded daily using Thermometers (ThermoPro[®] TP50 Digital LCD Thermometer, UAE) and Thermohygrometer (Digital Thermometer Hygrometer Indoor Outdoor Temperature Meter Humidity Monitor with LCD Alarm Clock, 3M Probe Cord, UAE), respectively.



Figure 1: Geographical map for Damietta governorate, Egypt (In: Elgammal M, Ali RR, Samra RA. Assessing of heavy metal pollution in soils of Damietta governorate, Egypt. International Conference on Advances in Agricultural, Biological and Environmental Sciences (AABES-2014) Oct 15-16, 2014 Dubai).

PREVALENCE OF EXTERNAL PARASITE INFESTATIONS

Point prevalence rates (PP) of parasitic infestations were calculated according to Thrusfield and Christley (2018) and Thrusfield (2007). The Point prevalence rates (PP) of the total and variables-specific parasitic infestations were calculated as follows:

Point prevalence (PP) rate =
$$(\alpha / \mu) \times 100$$

Where α is the number of (total/variable-specific) infested dogs and cats during a specific period and μ is the number of susceptible populations of dogs and cats during the same period. The variables-specific point prevalence was calculated concerning age, sex, breed, coat color, feed, housing system, and housing pattern.

SAMPLING

A total of 742 external parasites were collected during the study. The samples included 160 ticks (38 in fall, 20 in winter, 43 in spring, and 59 in summer), 380 fleas (176 from dogs; 50 in fall, 46 in winter, 52 in spring, and 28 in summer, and 204 from cats; 48 in fall, 55 in winter, 64 in spring, and 37 in summer), 5 lice (3 in fall and 2 in winter),

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45 skin mites (16 in fall, 4 in winter, 14 in spring, and 11 in summer, and 152 ear mite (44 in fall, 34 in winter, 40 in spring, and 34 in summer) samples. The samples were collected in sterile screw-capped bottles, labeled, and transferred to the laboratory in a dry-ice box as quickly as possible.

PARASITOLOGICAL ELCTRON EXAMINATIONS

The collected external parasite samples were subjected to fixation in equal volumes of glutaraldehyde 4% and cacodylate 0.2% for two hours as described by Farid et al. (2021). The samples were then washed in equal volumes of sucrose 0.4% and cacodylate 0.2% for additional two hours. Later, the samples were post-fixed in equal volumes of osmic acid 2% and cacodylate 0.3 % for one hour. The samples were washed with distilled water, dehydrated in ascending grades of ethyl alcohol for 5 min each (30%, 50%, 70%, and 90%), and mounted in absolute alcohol 100% for 10 min for 3 times (Aboelela et al., 2022). Specimens were glued by their dorsal and ventral surfaces to the SEM stub and were dried by the dryer (Blazer union, F1-9496 Blazer/Furstentun Leishtenstein) using liquid carbon dioxide. Specimens mounted on SEM stubs were coated with gold using SI5OA sputter coater and then examined by scanning electron microscopy (JSM-IT100 InTouchScope[™] Scanning Electron Microscope, JOEL, Damansara, Selangor, Malaysia) as recommended by Atteya et al. (2019).

STATISTICAL ANALYSIS

The statistical analysis was conducted using a statistical package for social sciences version 20 (IBM Corp, 2016 - IBM SPSS Statistics 20). The obtained data and results were analyzed statistically using multifactorial Analysis of Variance (ANOVA) to estimate the influence of seasonal variation on infestation rates in dogs and cats with concern to some factors such as breed, sex, age, coat color, housing system, housing pattern, and type of food. The statistical model empathized as follows:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ijk}$$

Where Y_{ijk} was the measurement of dependent variables; μ was the overall mean; α_i was the fixed effect of the different treatments (seasons), β_j was the fixed effect of environmental and host determinants, $(\alpha\beta)_{ij}$ was the interactions of seasons by determinant variation, and \mathcal{E}_{ijk} was the random error. Nonparametric Kruskal–Wallis was used for determining the significant differences between the reduction percentages. The results were expressed as highly significant at (P ≤ 0.01), significant at (P ≤ 0.05), and non-significant at (P > 0.05).

RESULTS AND DISCUSSION

AMBIENT ENVIRONMENTAL CONDITIONS

The ambient environmental conditions during the four seasons were fluctuating around the normal values. We recorded a temperature and RH up to 24.3°C and 59%, 18.3°C and 63%, 23.7°C and 53%, and 30.6°C and 56% during fall, winter, spring, and summer, respectively.

ECTOPARASITE INFESTATION RATES

The clinical examination revealed a total of 313 (20.7%) infested (Fall= 79, winter= 58, spring= 93, and summer= 83) out of 1511 admitted (Fall=269, winter=521, spring=409, and summer=312) dogs and 315 (29.2%) infested (Fall= 80, winter= 78, spring= 93, and summer= 64) out of 1078 admitted (Fall= 313, winter= 379, spring= 364, and summer= 64) cats.

POINT PREVALENCE AND ASSOCIATIONS OF ECTOPARASITE INFESTATIONS

Point prevalence of parasitic infestations revealed in Table 1 highly significant (P < 0.01) increases in dogs during fall, summer, spring, and winter, respectively. While in cats (Table 1), the higher PP_{infestation} was recorded with highly significant (P < 0.01) increases during spring and fall with no significant differences between the two seasons compared to winter and summer, respectively.

Age-specific $PP_{infestation}$ in dogs (Table 2) revealed highly significant (P < 0.01) increases during fall and spring with no significance in between, spring and summer with no

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significance in between, winter, and summer in dogs of <1 year, 1: 2 years, 2: 3 years, and <4 years respectively. On the other hand, high significant (P < 0.01) increases were recorded in PP_{infestation} at <1-year dogs during the four seasons of the study. Cats (Table 2) revealed highly significant (P < 0.01) increases during summer, spring, spring, and summer with no significant differences between, fall, winter, spring and summer with no significant differences between the four seasons, and summer in dogs of <1 year, 1: 2 years, 2: 3 years, 3: 4 years, and <4 years, respectively. On the other hand, highly significant (P < 0.01) increases were recorded in PP_{infestation} at < 1-year cats during the four seasons of the study.

Table 1: Point prevalence (PP) of parasitic infestation indogs and cats concerning seasonal variation.

Seasons		Dog	6	Cats					
	Adm.	Infes.	PP (%)	Adm.	Infes.	PP (%)			
Fall	269	79	29.37ª	313	80	25.56ª			
Winter	521	58	11.13 ^d	379	78	20.58 ^b			
Spring	409	93	22.74°	364	93	25.54ª			
Summer	312	83	26.60 ^b	337	64	18.99°			
P-value			0.002			0.014			

Means carrying different superscripts in the same column are significantly different at ($P \le 0.05$) or highly significantly different at (P < 0.01). Means carrying the same superscripts in the same column are non-significantly different at (P < 0.05). Adm= Admitted cases, Infes= Infested cases, PP= Point prevalence.

Species	Age/Y	Seasons									
			Fall		Winter		Spring		Summer		
		No	PP%	No	PP%	No	PP%	No	PP %		
Dogs	<1 y	61	77.21 ^{Aa}	45	77.58^{Aa}	68	73.12^{Ba}	60	72.28^{Ba}	0.022	
	1: 2 y	13	16.45 ^{Bb}	9	11.39 ^{Cb}	20	21.51 ^{Ab}	18	21.68 ^{Ab}	0.008	
	2: 3 y	3	3.79 ^{Bc}	3	5.17 ^{Ac}	1	1.07 ^{Cd}	0	0.00^{Dd}	0.006	
	3: 4 y	0	0.00 ^{Ad}	0	0.00 ^{Ae}	0	0.00 ^{Ae}	0	0.00 ^{Ad}	0.002	
	>4 y	2	2.53 ^{Cc}	1	1.72 ^{Cd}	4	4.30 ^{Bc}	5	6.02 ^{Ac}	0.007	
	P-value		0.019		0.005		0.000		0.008		
Cats	<1 y	59	73.75^{Ba}	54	69.23 ^{Ca}	57	61.29 ^{Da}	48	75.00^{Aa}	0.002	
	1: 2 y	12	15.00 ^{Cb}	17	21.79 ^{Bb}	22	23.66 ^{Ab}	5	7.81 ^{Dc}	0.001	
	2: 3 y	3	3.75^{Bd}	2	2.56^{Bd}	6	6.45 ^{Ac}	4	6.25 ^{Ad}	0.013	
	3: 4 y	1	1.25 ^{Ae}	1	1.28 ^{Ad}	2	2.15 ^{Ac}	1	1.56 ^{Ae}	0.422	
	>4 y	5	6.25 ^{Bc}	4	5.13 ^{Bc}	6	6.45^{Bd}	6	9.38 ^{Ab}	0.009	
	P-value		0.000		0.002		0.001		0.000		

Table 2: Age-specific point prevalence (PP) of parasitic infestation in dogs and cats concerning seasonal variation.

^{A, B, C, and D} Means carrying different superscripts in the same row are significantly different at ($P \le 0.05$) or highly significantly different at ($P \le 0.05$). ^{a,b,c,d, and,e} Means carrying different superscripts in the same column are significantly different at ($P \le 0.05$) or highly significantly different at (P < 0.05). ^{a,b,c,d, and,e} Means carrying different superscripts in the same column are significantly different at ($P \le 0.05$) or highly significantly different at (P < 0.01). Means carrying the same superscripts in the same column are non-significantly different at (P < 0.05). P: Point prevalence. Infested dogs were in fall= 79, winter= 58, spring= 93, summer= 83. Infested cats were in fall= 80, winter= 78, spring= 93, summer= 64.

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Sex-specific PP_{infestation} in Table 3 revealed in dogs highly significant (P < 0.01) increases during winter in males and spring in females. While in cats highly significant (P < 0.01) increases in PP_{infestation} were recorded during summer in males and winter in females (Table 3).

Breed-specific $PP_{infestation}$ in dogs (Table 4) revealed highly significant (P < 0.01) increases during spring and summer with no significant differences, fall and winter with no significant differences, spring, summer, winter and spring, winter, and Fall in German and Husky, Rottweiler, Toy,

Golden, Pit Bull and Boxer, Milionis, and Native and others breeds respectively. From the other view, German dogs revealed highly significant (P < 0.01) increases in PP_{infestation} in the four seasons of the study compared to other breeds. Cats (Table 4) revealed highly significant (P < 0.01) increases during winter, the four seasons with no significant differences in between, fall in Persian, Siamese and Moa, and Native; Himalaya and Mix breeds respectively. On the other hand, Persian cats revealed highly significant (P < 0.01) increases in PP_{infestation} in the four seasons of the study compared to other breeds.

Table 3: Sex-specific point prevalence (PP) of parasitic infestation in dogs and cats concerning seasonal variation.

Seasons		Dogs			Cats						
	Males			Females		Males		Females			
	No	PP %	No	PP %	No	PP %	No	PP %			
Fall	49	62.03 ^b	30	37.97 ^b	42	52.50^{b}	38	47.50 ^c			
Winter	38	65.52ª	20	34.48 ^c	29	37.18^{d}	49	62.82ª			
Spring	56	60.22 ^c	37	39.78ª	46	49.46 ^c	47	50.54 ^b			
Summer	51	61.45 ^{bc}	32	38.55 ^b	39	60.94ª	25	39.06 ^d			
P-value		0.012		0.009		0.000		0.000			

Means carrying different superscripts in the same column are significantly different at ($P \le 0.05$) or highly significantly different at (P < 0.01). Means carrying the same superscripts in the same column are non-significantly different at (P < 0.05). PP= Point prevalence. Infested dogs were in fall= 79, winter= 58, spring= 93, summer= 83. Infested cats were in fall= 80, winter= 78, spring= 93, summer= 64.

Species	Breeds	Seasons											
-			Fall		Winter		Spring		Summer				
		No	PP%	No	PP%	No	PP%	No	PP%				
Dogs	German	22	27.85 ^{Ca}	18	31.03^{Ba}	40	43.01 ^{Aa}	37	44.58 ^A	0.009			
	Rottweiler	4	5.06^{Ad}	2	3.45^{Af}	1	1.08^{Bg}	1	1.20^{Bg}	0.017			
	Husky	2	2.53 ^{Be}	1	1.72^{Bg}	3	3.23 ^{Ae}	3	3.61 ^{Ae}	0.013			
	Toy	18	22.78 ^{Bb}	13	22.41 ^{Bb}	22	23.66 ^{Ab}	11	13.25 ^{Cb}	0.008			
	Golden	4	5.06^{Bd}	1	1.72 ^{Cg}	2	2.15 ^{Cf}	6	7.23 ^{Ac}	0.009			
	Pit Bull	4	5.06^{Bd}	7	12.07 ^{Ad}	9	9.68 ^{Ad}	5	6.02^{Bd}	0.015			
	Boxer	2	2.53 ^{Be}	2	3.45^{Af}	3	3.23 ^{Ae}	0	0.00^{Ch}	0.007			
	Milionis	2	2.53 ^{Be}	5	8.62 ^{Ae}	0	0.00^{Dh}	1	1.20 ^{Cg}	0.005			
	Native	4	5.06^{Ad}	1	1.72 ^{Cg}	2	2.15^{Bf}	2	2.41^{Bf}	0.014			
	Others	17	21.52 ^{Ac}	8	13.79 ^{Cc}	11	11.83 ^{Dc}	17	20.48^{Ba}	0.004			
	P-value		0.004		0.001		0.000		0.000				
Cats	Persian	57	71.25^{Da}	74	94.87 ^{Aa}	87	93.55^{Ba}	55	85.94 ^{Ca}	0.001			
	Siamese	0	0.00^{Ad}	0	0.00^{Ad}	0	0.00 ^{Ad}	0	0.00 ^{Ae}	0.432			
	Native	3	3.75 ^{Ac}	2	2.56 ^{Bb}	0	0.00^{Dd}	1	1.56 ^{Cd}	0.019			
	Himalaya	3	3.75 ^{Ac}	1	1.28^{Bc}	1	1.08^{Bc}	2	3.13 ^{Ac}	0.008			
	Moa	0	0.00^{Ad}	0	0.00^{Ad}	0	0.00^{Ad}	0	0.00^{Ae}	0.546			
	Mix	17	21.25 ^{Ab}	1	1.28 ^{Dc}	5	5.38 ^{Cb}	6	9.38 ^{Bb}	0.001			
	P-value		0.001		0.002		0.000		0.001				

Table 4: Breed-specific point prevalence (PP) of parasitic infestation in infested dogs and cats concerning seasonal variation.

^{A, B, C, and D} Means carrying different superscripts in the same row are significantly different at ($P \le 0.05$) or highly significantly different at ($P \le 0.05$). ^{a,b,c,d, and,e} Means carrying different superscripts in the same column are significantly different at ($P \le 0.05$) or highly significantly different at (P < 0.05). ^{a,b,c,d, and,e} Means carrying different superscripts in the same column are significantly different at ($P \le 0.05$) or highly significantly different at (P < 0.05). ^{a,b,c,d, and,e} Means carrying the same superscripts in the same column are non-significantly different at (P < 0.05). P: Point prevalence. Infested dogs were in fall= 79, winter= 58, spring= 93, summer= 83. Infested cats were in fall= 80, winter= 78, spring= 93, summer= 64.

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Coat color-specific $PP_{infestation}$ (Table 5) in dogs revealed highly significant (P < 0.01) increases during summer, winter, fall and summer, fall, fall and spring, and winter seasons in black and tan, black, golden, tan, brindle, and white, others coated dogs, respectively. On an overall means, $PP_{infestation}$ revealed highly significant (P < 0.01) increases during fall, spring and summer in black and Tan, and winter in other coated dogs. Coat color-specific

 $PP_{infestation}$ (Table 5) in cats revealed highly significant (P < 0.01) increases during spring, winter and summer, fall and spring, spring and summer, and fall in white, blue, red, cream, and mixed respectively. Coat color-specific $PP_{infestation}$ also revealed highly significant (P < 0.01) increases in mixed coated during fall, winter and summer and white-coated cats during spring.

Table 5: Coat color-specific point prevalence (PP) of parasitic infestation in infested dogs and cats concerning seasonal variation.

Species	Breeds	Seasons											
			Fall		Winter		Spring	ç	Summer				
		No	PP%	No	PP%	No	PP%	No	PP%				
Dogs	Black- Tan	30	37.97 ^{Ca}	19	32.76^{Db}	39	41.94^{Ba}	38	45.78 ^A	0.001			
	Black	2	2.53 ^{Ce}	7	12.07^{Ad}	5	5.38 ^{Bd}	4	4.82 ^{Bd}	0.008			
	Golden	6	7.59^{Ad}	2	3.45 ^{Be}	2	2.15^{Be}	7	8.43 ^{Ac}	0.012			
	Tan	7	8.86 ^{Ad}	0	0.00^{Df}	5	5.38 ^{Bd}	1	1.20 ^{Cf}	0.002			
	Brindle	5	6.33 ^{Ad}	2	3.45 ^{Be}	5	5.38 ^{Ad}	3	3.61 ^{Be}	0.007			
	White	12	15.19 ^{Ac}	8	13.79^{Bc}	15	16.13 ^{Ac}	10	12.05 ^{Bb}	0.012			
	Others	17	21.52 ^{Cb}	20	34.48^{Aa}	22	23.66 ^{Bb}	20	24.10^{Ba}	0.009			
	P-value		0.001		0.000		0.002		0.001				
Cats	White	19	23.75 ^{Bb}	14	17.95 ^{сь}	29	31.18 ^{Aa}	15	23.44 ^{Bb}	0.009			
	Blue	4	5.00 ^{Cd}	9	11.54^{Ad}	7	7.53 ^{Be}	8	12.50 ^{Ae}	0.012			
	Red	17	21.25 ^{Ac}	12	15.38^{Bc}	19	20.43 ^{Ac}	13	20.31 ^{Ac}	0.015			
	Cream	1	1.25 ^{Ce}	9	11.54^{Bd}	13	13.98 ^{Ad}	9	14.06^{Ad}	0.007			
	Mixed	39	48.75 ^{Aa}	34	43.59^{Ba}	25	26.88 ^{Db}	19	29.69 ^{Ca}	0.002			
	P-value		0.000		0.001		0.000		0.001				

^{A, B, C, and D} Means carrying different superscripts in the same row are significantly different at ($P \le 0.05$) or highly significantly different at ($P \le 0.05$). ^{a,b,c,d, and, e} Means carrying different superscripts in the same column are significantly different at ($P \le 0.05$) or highly significantly different at (P < 0.05). ^{a,b,c,d, and, e} Means carrying different superscripts in the same column are significantly different at ($P \le 0.05$) or highly significantly different at (P < 0.01). Means carrying the same superscripts in the same column are non-significantly different at (P < 0.05). PP: Point prevalence. Infested dogs were in fall= 79, winter= 58, spring= 93, summer= 83. Infested cats were in fall= 80, winter= 78, spring= 93, summer= 64.

Table 6: Housing-specific p	point prevalence ((PP) o	of parasitic	infestation	in i	infested	dogs a	and cats	concerning	seasonal
variation.										

Species	Seasons		Housing	systems		Housing patterns				
		In-door		Out-door		Single		Multiple		
		No	PP%	No	PP%	No	PP%	No	PP%	
Dogs	Fall	23	29.11ª	56	70.89 ^b	49	62.03 ^d	30	37.97ª	
	Winter	11	18.97°	47	81.03ª	44	75.86 ^b	14	24.14 ^c	
	Spring	21	22.58 ^b	72	77.42^{b}	61	65.59°	32	34.41 ^b	
	Summer	19	22.89 ^b	64	77.11 ^b	68	81.93ª	15	18.07 ^d	
	P-value		0.005		0.002		0.000		0.000	
Cats	Fall	75	93.75°	5	6.25 ^b	53	66.25°	27	33.75 ^b	
	Winter	76	97.44 ^b	2	2.56°	54	69.23 ^b	24	30.77°	
	Spring	84	90.32 ^d	9	9.68ª	60	64.52 ^d	33	35.48ª	
	Summer	63	98.44ª	1	1.56 ^d	46	71.88ª	18	28.13 ^d	
	P-value		0.000		0.000		0.000		0.001	

Means carrying different superscripts in the same column are significantly different at ($P \le 0.05$) or highly significantly different at (P < 0.01). Means carrying the same superscripts in the same column are non-significantly different at (P < 0.05). PP: Point prevalence. Infested dogs were in fall= 79, winter= 58, spring= 93, summer= 83. Infested cats were in fall= 80, winter= 78, spring= 93, summer= 64.

Table 7: Food-specific point prevalence (PP) of parasitic infestation in infested dogs and cats concerning seasonal variation.

Species	Seasons	Food varieties										
		Dry	Dry		ed	Raw		Cann	ed	Mix		
		No	PP%	No	PP%	No	PP%	No	PP%	No	PP%	
Dogs	Fall	2	2.53 ^b	48	60.76ª	13	16.46°	0	0.00^{b}	16	20.25 ^b	
	Winter	2	3.45 ^b	31	53.45°	15	25.86ª	1	1.72ª	9	15.52°	
	Spring	5	5.38ª	57	61.29ª	9	9.68 ^d	0	0.00^{b}	22	23.66ª	
	Summer	1	1.20 ^c	47	56.63 ^b	17	20.48 ^b	0	0.00^{b}	18	21.69 ^b	
	P-value		0.008		0.005		0.000		0.004		0000	
Cats	Fall	4	5.00 ^d	23	28.75 ^b	0	0.00^{b}	0	0.00ª	53	66.25ª	
	Winter	7	8.97 ^b	33	42.31ª	0	0.00^{b}	0	0.00ª	38	48.72 ^b	
	Spring	10	10.75ª	40	43.01ª	1	1.08ª	0	0.00ª	42	45.16 ^c	
	Summer	5	7.81°	28	43.75ª	0	0.00^{b}	0	0.00ª	31	48.44 ^b	
	P-value		0.000		0.007		0.005		0.471		0.012	

Means carrying different superscripts in the same column are significantly different at ($P \le 0.05$) or highly significantly different at ($P \le 0.01$). Means carrying the same superscripts in the same column are non-significantly different at (P < 0.05). PP: Point prevalence; Infested dogs were in fall= 79, winter= 58, spring= 93, summer= 83. Infested cats were in fall= 80, winter= 78, spring= 93, summer= 64.

Housing system-specific PP_{infestation} in dogs revealed in Table 6 highly significant (P < 0.01) increases during fall in the indoor housing system and winter in the outdoor housing system. Housing pattern-specific PP_{infestation} in dogs revealed in Table 6 highly significant (P < 0.01) increases during summer in the single and fall in the multiple housing system. Cats revealed in Table 6 highly significant (P < 0.01) increases during summer in the indoor housing system and spring in the outdoor housing system. Housing pattern-specific PP_{infestation} in cats revealed in Table 6 highly significant (P < 0.01) increases during summer in the single and spring in the multiple housing system.

Food-specific $PP_{infestation}$ in dogs revealed in Table 7 highly significant (P < 0.01) increases during spring, spring, winter, winter, and spring seasons in dogs consumed dry, cooked, raw, canned, and mixed food respectively. Meanwhile, food-specific $PP_{infestation}$ in cats revealed in Table 7 highly significant (P < 0.01) increases during spring, spring and summer, spring, and fall seasons in cats consumed dry, cooked, raw, and mixed food, respectively.

Species-specific $PP_{infestation}$ in dogs revealed in Figure 2A highly significant (P < 0.01) increases in infestations with fleas during spring, ticks during summer, and skin mites and lice during fall. Meanwhile, species-specific $PP_{infestation}$ in cats revealed in Figure 2B highly significant (P < 0.01) increases in infestations with fleas during spring and ear mites during the fall season.



Figure 2: Species-specific point prevalence of different external parasites infesting dogs and cats concerning seasonal variation. **A:** External parasites infesting dogs. **B:** External parasites infesting cats.

ELECTRON MICROSCOPIC PARASITOLOGICAL EXAMINATIONS

Rhipicephalus sanguineus (Latreille, 1806) is characterized by a hexagonal shape basis capitulum. The palpi consist of four segments, with the first three ones being larger than the fourth, which occupies a cavity at the ventral surface

of the third segment. Palpi were short and not ridged dorsally and laterally. The fourth palpi segment has an earlike shape. This tick species was unornamented except for the presence of some ornamentation on the chelicerae. The cheliceral base is more bulged and has a pyramidal shape. The Porose area was nearly triangular with a lot of smooth setae. The hypostome dentation was 3/3 and there are a lot of small spines in the anterior end of the hypostome. There are smooth setae or setae with one or various rows of denticles along their length arranged in two rows (12 pairs in number). There were fingerprints like projections on the body's surface. Their eyes were convex and not depressed. The spiracular plate was comma-shaped. Males have a pair of adenal shields (large adenal shields, less acute anteriorly, only slightly widened, and somewhat angular posteriorly) and accessory shields. Festoons were delimitated only by deep lateral grooves, presence of caudal appendages and distinct anal groove, cervical fields texture has wrinkled areas, genital aperture posterior lips have abroad U shape (Figure 3).



Figure 3: Scanning Electron microscopic picture of A: *Rhipicephalus sanguineus* ventral view, B: *Rhipicephalus sanguineus* dorsal view, C: Ventral view showing pedipalps and toothed hypostome, and D: Dorsal view showing hexagonal dorsal basis capituli.

Advances in Animal and Veterinary Sciences The insects (Flea in Figure 4 and Lice in Figure 5) showed that the dorsal surface is covered with long and short smooth setae directed backward.





Figure 4: Scanning Electron microscopic picture of A: *Ctenocephalides felis* female, B: *Ctenocephalides felis* male, C: Spine 1 of genal ctenidium nearly equal to spine 2, D: Pronotal comb, and E: Posterior end male.

The mites showed the dorsal surface of both ear mite and *Sarcoptes* mite covered with several folds and grooves, the cuticle finely striated, several fingerprints like projections appear on the body surface, There are small spines with various rows directed backward, in addition to finally smooth setae (Figures 6 and 7).

Ectoparaisitic infestations in animals are dependent on some host determinants such as species, animal density, host range, breed, age, sex, behavior, and skin covering (Pakdad et al., 2012; Földvári et al., 2016), environmental determinants such as temperature, relative humidity, dew point, wind flow, location, and seasonal variations (Benedek et al., 2011), and agent determinants such as nutritional, developmental, and maturation requirements (Moravvej et al., 2015). Dogs and cats' ectoparasites have been classified into Acarina (tick), Siphonaptera (fleas), Mesostigmata (mite), and Phthiraptera (lice). Fleas can act as a biological vector for some microbial agents such

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as Yersinia pestis, Borrelia, Salmonella, Francisella tularensis, Trypanosoma, and Leishmania (Kwak, 2017). The mite has been known to parasitize and transmit Wuchareria bancrofti (Harrison et al., 2015). Lice are also known for their ability to transmit plague and Rickettsia typhi (Frye et al., 2015).



Figure 5: Scanning Electron microscopic picture of A: *Heterodoxus spiniger* male, B: *Heterodoxus spiniger* female, C: Posterior end female, and D: Posterior end male.

Control and eradication strategies of external parasites have been included in every single plan for biosecurity on all animals and pet animals' farms (Alho et al., 2018). The control actions depend on sealing with cement all the cracks in the walls and floors of the animals' building, good housing practices, and design, rotational grassing to encourage killing the larvae from starvation, depopulation if possible, washing of the animals routinely, and application of chemical or biological treatment means on the animals (Cochi et al., 1998).



Figure 6: Scanning Electron microscopic picture of A: *Otodectes cynotis*, anterior end male, B: *O. cynotis*, posterior end male, C: anterior legs of *O. cynotis* male, D: finger-like projections present on the dorsum of *O. cynotis*, E: posterior legs of *O. cynotis* male.

The current study showed that PP_{infestations} revealed highly significant increases in dogs during fall and in cats during spring and fall with no significant differences between the two seasons. Palmer et al. (2010) and Shoorijeh et al. (2008) in agreement with our results documented that infestation happened mainly during springtime concerning the increased temperature and decreased humidity significantly increased parasitic infestation. They assed that summertime and springtime showed a significant increase in the number of animals with external parasite infestation when compared with wintertime. El-Seify et al. (2016) revealed that the seasonal prevalence reached 100% during summer and decreased to 95% during autumn, and 80% during spring, and the most significant decrease appeared during winter with 50%.

Age-specific $PP_{infestations}$ of dogs in the current study revealed highly significant increases during fall and spring, spring and summer, winter, and summer in dogs of <1 year, 1: 2

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years, 2: 3 years, and <4 years respectively. Cats revealed highly significant increases during summer, spring, spring, and summer with no significant differences between, fall; winter; spring and summer with no significant differences between the four seasons, and summer in dogs of <1 year, 1: 2 years, 2: 3 years, 3: 4 years, and <4 years respectively. From another point of view, highly significant (P < 0.01) increases were recorded in $\mathrm{PP}_{_{\mathrm{infestations}}}$ at < 1-year dogs and cats during the four seasons of the study. The results were consistent with those of Pereira et al. (2016) and El-Seify et al. (2016) who recorded that cats with body weight <1.4 kg showed to some degree lower prevalence of 75% when compared with cats with body weight >1.5 kg. Cats under or/at one year old showed a lower infestation level of 81.3% when compared with 87% in cats above one year of age. Kumsa et al. (2019) also reported that Ixodida (Siphonaptera) showed significantly elevated levels in younger in comparison with older cats.



Figure 7: Scanning Electron microscopic picture of A: Sarcoptes scabiei, anterior end, B: S. scabiei, posterior end female, C: The un-segmented pedicels of anterior legs of S. scabiei female are terminate by a disk-like structure, D: Thorn like spines on the dorsum of Sarcoptic mite.

Sex-specific $PP_{infestations}$ in the current study revealed highly significant increases during winter in males and spring in female dogs, and during summer in males and winter in female cats. Moskvina and Zhelenznova (2015) mentioned that sex did not cause a significant difference in the prevalence of *Otodectes cynotis* in canines or felines. Abuzeid (2015) reported that *Heterodoxus spiniger* showed a significant increase in younger dogs when compared with older ones, while *Hippobosca longipennis* showed a significant increase in older dogs when compared with younger ones and sex did not affect the prevalence of external parasites.

The current study showed that breed-specific $PP_{infestations}$ revealed highly significant increases in German dogs and Persian cats during the four seasons of the study compared to other dogs' and cats' breeds. The results were partially consistent with those of Hasib et al. (2020) who involved 845 animals (488 dogs and 361 cats) in a retrospective study. Cats external parasite infestation showed no significant difference in different seasons, breeds, ages, or genders. While dogs' infestation showed a statistically significant difference in the rainy season and no statistical difference among breeds, ages, or genders.

Coat color-specific PP_{infestations} revealed highly significant increases during fall, spring and summer in black and Tan, and winter in other coated dogs. While in cats highly significant increases were recorded in mixed coated during fall; winter and summer and white-coated cats during spring. The results were synchronized with those of Bahrami et al. (2012) who stated that dogs possessing darker hair tone (black) showed higher levels of infestation in comparison with lighter hair tone dogs. Also, Abdulkareem et al. (2019) and Otranto et al. (2017) reported that females and animals of young age had significantly elevated levels of infestation and added that fur coloration and breed did not affect infestation significantly.

Dogs' housing system-specific $PP_{infestations}$ revealed highly significant increases during fall in the indoor housing system and winter in the outdoor housing system, while housing pattern-specific PP_{infestations} revealed highly significant increases during summer in the single and fall in the multiple housing system. Cats' external parasite infestation revealed highly significant increases during summer in the indoor housing system and spring in the outdoor housing system, while housing pattern-specific PP_{infestations} revealed highly significant increases during summer in the single and spring in the multiple housing system. The results were compatible with those of Memon et al. (2018) and Ferreira et al. (2017) who stated that the most elevated PP of parasitic infestations' existence was recorded in June with a percentage of 30.86%. Outdoor dogs' external parasite infestation showed a significant statistical increase in comparison to indoor ones.

Food-specific PP_{infestations} revealed highly significant increases during spring, spring, winter, winter, and spring seasons in dogs consumed dry, cooked, raw, canned, and mixed food respectively in dogs, and during spring, spring and summer, spring, and fall seasons consumed dry, cooked, raw, and mixed food, respectively in cats. On the

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contrary, Minabaji et al. (2020) showed that gender and housing significantly affected external parasite infestation while neither life stage (age), coat color, food type, year division, or fur extent affected external parasite infestation significantly.

Species-specific PP_{infestations} of dogs revealed highly significant increases in fleas infestations during spring, tick during summer, and skin mites and lice during fall. Meanwhile, species-specific PP_{infestations} of cats revealed highly significant increases in fleas infestation during spring and ear mite during the fall season. Niche used an epidemiological base to explain the ecological distribution of parasitic infestation and microbial infections, as well as Alho et al. (2017); Hamidi et al. (2015); Dziemian et al. (2014) explained that the moderate microclimatic conditions and food availability in the area which mostly prevail in summer and spring seasons encourage the growth and multiplication of ectoparasites.

The current study identified from the parasitological examinations the following external parasites: Rhipicephalus sanguineus ticks, Ctenocephalides canis flea, Heterodoxus spiniger lice, and Sarcoptic scabiei mites in dogs, Otodectes cynotis ear mites and Ctenocephalides felis flea in cats. The results were synchronized with those of Xhaxhiu et al. (2009) who inspected 181 canine pets and 26 shorthair cats in the suburb near Tirana, Albania for the existence of external parasites. The canines were inspected on various junctures: wintertime (Dec- Feb), springtime (Mar-May), and summertime (June-Aug) from the year 2005 to the year 2009 while the feline was inspected on the belated autumntime (Nov). The levels of external parasitism of canines are Rhipicephalus sanguineus with a percentage of 28.3%. Ixodes ricinus with a percentage of 0.6%. Sarcoptes scabiei var. Canis with the percentage of 4.4%, Otodectes cynotis with the percentage of 6.7%, Demodex canis with the percentage of 0.6%, Ctenocephalides canis with the percentage of 75.7%, Ctenocephalides felis with the percentage of 5%, Pulex irritans with the percentage of 8.3%, and Trichodectes canis with the percentage of 6.6%. Dogs infested with 2-3 different types of external parasites represented 38.1% of infested dogs. Inspected cats were found to be infested with a single type of external parasite which is *Ctenocephalides felis*. Also, Memon et al. (2018) inspected 150 cats (90 males and 60 females) for external parasites' existence in Karachi city. Rhipicephalus sanguineus was the prevalent external parasite with a percentage of 39.33%, C. canis and Demodex canis with a percentage of 4.67%, Dermacentor reticulates and Trichodectes canis with a percentage of 4%, and *C. felis* with the percentage of 1.33%.

Tamarat et al. (2019) also inspected 384 dogs for external parasites' existence. 95% showed infestation with sole or combined types of the 7 detected external parasites. The

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descending order of infestation was 79.69% with C. felis, 71.35% with C. canis, 10.42% with R. sanguineus, and 7.81% with Lingonathus setosus, 4.17% with P. irritans, 2.6% with T. canis, and 2.6% with Ambylomma species. Lefkaditis et al. (2016) reported that younger animals showed significantly elevated levels of infestation in comparison with older ones reaching adulthood, and male animals showed significantly elevated levels of infestation in comparison with female animals. Meanwhile shorter hair and smaller sized animals showed a non-significant disparity in comparison with longer hair and larger sized animals. Ebrahimzade et al. (2016) inspected 70 dogs for the existence of external parasites. The amount of infested animals represented 100% in the area of Mazandaran province, 68.5% in the area of Gilan province, and 93.3% in Qazvin province. Flea was found in 77.5% of infested animals. Lice were found in fifty percent, ticks were found in 8.6%, flies were found in 6.8%, and mites were found in 5.1%. Four types of fleas were recorded including Ctenocephalides canis with a percentage of 29.8%, C. felis with a percentage of 19.9%, Pulex iritans with a percentage of 2.9%, and Xenopsiella cheopis with a percentage of 0.7%. One type of lice was found which is *Trichodectes canis* with a percentage of 41.3%, one type of ticks was found which is Rhipicephalus sanguines with a percentage of 0.7%, and one type of flies was found which is Hippobosca sp. with the percentage of 1.1%, and one type of mites was found which is Sarcoptes scabiei with the percentage of 3.6%.

Minabaji et al. (2020) inspected 460 dogs for external parasite existence 99 animals happened to show infestation representing 21.52%. C. *canis* and *Pulex irritans* infestation represent the percentage of 10.43%, *Rhipicephalus turanicus* infestation represents the percentage of 3.04%, *Sarcoptes scabiei* infestation represents the percentage of 2.7%, *Hippobosca longipennis* infestation representing the percentage of 2.7%, *Rhipicephalus sanguineus* infestation representing the percentage of 1.95%, *Wohlfahrtia magnifica* infestation representing the percentage of 1.95%, *Demodex canis* infestation representing the percentage of 0.65%, *Otodectes cynotis* infestation representing the percentage of 0.43%, *Haemaphysalis ernacei* infestation representing the percentage of 0.21%, and *Linognathus setosus* infestation representing the percentage of 0.21%.

CONCLUSIONS AND RECOMMENDATION

Dogs and cats are the most important human companion pets that are susceptible to the danger of external parasite infestation. PP_{infestation} of dogs and cats in the current study showed strong associations with the animal determinants such as species (dogs and cats), breed (German, Rottweiller, Husky, Toy, Golden, Pit Bull, Boxer, Milionis, and other

dogs; Persian, Native, Himalaya, Moa, and Mix cats), sex (males and females), age (<1, 1: 2, 2: 3, 3: 4, and >4 years), and coat color (Black-tan, black, golden, tan, brindle, white, and others in dogs; white, blue, red, cream, and mixed in cats). PP_{infestation} of dogs and cats in the current study also showed strong associations with the environmental determinants such as housing system (in-door and outdoor) and pattern (single and multiple) and type of food (dry, cooked, raw, canned, and mixed), as well as with the type of infesting external parasites concerning seasonal variations.

The parasitological examinations identified some external parasites as follows: *Rhipicephalus sanguineus* ticks, *Ctenocephalides canis* flea, *Heterodoxus spiniger* lice, and *Sarcoptic scabiei* mites in dogs, *Otodectes cynotis* ear mites, and *Ctenocephalides felis* flea in cats.

Biosecurity measures should be considered with concern to all the physiological demands of dogs and cats. These measures include health status monitoring and reporting, proper design houses, routine examination, proper hygienic measures, feed and water of good quality, strategies of waste disposal, cleaning and disinfection procedures, medication and vaccination needs, and proper handling.

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NOVELTY STATEMENT

The study focused on the existence, distribution, and prevalence of the most predominant ectoparasitic infestation on human-companion pet animals (dogs and cats), as well as the parasitological identification of these ectoparasites using electron microscopic examination.

AUTHOR'S CONTRIBUTION

ESS designed the study designs, supervised the execution of the study, calculated the epidemiological prevalence rates, and took a part in writing the manuscript. EMA participated in the cross-sectional study, collected the external parasite samples, and took a part in writing the manuscript. EMA conducted the parasitological examinations and took a part in writing the manuscript. MAS participated in the cross-sectional study, participated in collecting the external parasite samples, and took a part in writing the manuscript.

The authors have declared no conflict of interest.

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