

Frequency, distribution, and molecular recognition of *Argas persicus* (Argasidae) infestation of local chicken *Gallus gallus domesticus* in Mosul city

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Abstract

Seven hundred sixty-four ticks of *Argas persicus* have been collected from 390 local chickens at four locations in Mosul city. Ticks have been determined by investigating their existence in different chicken cages. The raised distribution rates through the shelters in the wooden cage reached 61.9%, no presence of ticks appeared in the iron cage 0% as well as the total infestation rate with *A. persicus* in the shelter was 36.47%. The highest shelter infestation rate was from the eastern areas of the city 11.76%, although the lowest ratio was for those areas located in the west 6.47%. Moreover, the study showed statistical differences in frequency rates between summer 22.2% and autumn 10%. Tick frequency rates through July were elevated 33.4%, matching those for other summer and fall months 20.8, 29.7, 12.4, and 3.7%. A significant difference also could be seen in adult females' numbers and rates among the disparate months 25.3, 31.6, 34.5, 6.3, and 2.3%. The same applies regarding the nymphs and larvae of *A. persicus*, with significant differences by month in which the study was conducted for ticks' males; no noticeable differences have been shown among months of this work. Molecular analysis for 32 specimens of singular ticks was accustomed for extraction of DNA and restrained to PCR utilizing definite primers for exaggeration of sectional fragment of COX1, as well as 16SrRNA genes. All 32 isolates showed effective PCR results following gel electrophoresis with 240 bp and 606 bp product sizes.

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Introduction

Knowledge about species of tick's variety, and their geographical dissemination and frequency in the Asian continent, inclusive of China, India, Pakistan, Kazakhstan, Iran, and Iraq, is hard to come by and limits and make it encounter the domesticated fauna and wild ones, together with the possibility pathogens that it has the ability to transmit as vectors can significantly add to wild and captive animals' maintenance work (1). Little is known about the assortment of ectoparasites infesting the pasture-raised hens in Algeria. Soft ticks, especially *Argas persicus*, had been isolated through spring and summer, mostly from cracks and

services; at the same time, red mites of the chicken *Dermanyssus gallinae*, which had been observed in autumn, with great affluence of these parasites in chicken nests (2,3). The Migratory Birds Research Center, with the Institution of National Park Research in the Republic of Korea, collaborated to discover the Argasid ticks from two uninhabited islands in the Republic of Korea infested the Gull with a black tail and their nests, which had been seen dead. It was the first record of Argasid tick from this seabird breeding islands (4,5). Since the soft tick is a powerful vector of numerous diseases, detection of the tick species is clinically essential to save public health and deal with the veterinary troubles in the neighborhoods; among 1209

collected ticks, 8% of them were *Argas persicus* from poultry, which existed in the borderline of Iran-Iraq (6,7). And because it is known that the fowl tick *A. persicus* is an obligate ectoparasite of chicken, as well as the bad effects of this organism on birds productivity and may kill the birds because of heavy infestation and transmittable diseases like avian borreliosis, in Jeddah governorate, Saudi Arabia, two of researchers recorded several soft tick *A. persicus* in four chicken farms of five, they were found that there is a positive interaction between the average numbers of the fowl tick and the average on of the poultry in the time of their study, so they concluded that there a big problem in Baladi chicken farms in the governorate of Jeddah (8,9). Ectoparasites control is essential to maintain the farms biosafety. In contrast, the lack of a good understanding of control measurement led to a boost in the risk of chicken-related health problems and economic costs (10). It turned out that the burden of *A. persicus* was 3.3 per animal constantly, and a signal tick sucked 18.6 ml of blood every day, so more than 50 ml of the blood was sucked by tick's pair birds daily (11). The fact that some species share morphological characters although they have not been molecularly screened for a very long time previously (12), Whereabout, the basis of faulty taxonomic characterization or confusion involves the fields of inspection at different levels, so the soft tick of genus *Argas*, mainly *A. persicus* is a clear model of such mismatch: The explanation of this taxonomic unit has altered dramatically during the past decades (13). Lone ticks were used for extraction of DNA and exposed to PCR by applying definite primers for the exaggeration of a fractional particle of (COX1) as well as 16S r RNA genes, which have been necessary for verifying the detection of *A. persicus*, especially nowadays (14).

The objective of this work was to establish the distribution and frequency of *Argas persicus* on hosts (chicken) and their houses in the different regions of Mosul city from which the soft ticks were collected, especially during the months of the Summer and fall seasons, and to entrench the molecular recognition of soft tick *A. persicus* by PCR.

Materials and methods

Ethical approval

The conventional animal care and use consultants in the Veterinary Medicine College, University of Mosul, were ethically acceptable for this study (UM.VET.2023.100) on 21/6/2023.

Tick sources

Argas persicus were collected in 764 samples and were divided as follows: 172 males of *A. persicus*, 304 females, 96 nymphs, and 192 larvae, which were isolated from the chicken body specifically as devoid of feathers. Moreover, samples have been obtained from nests, edges, and corners

of the cages, cage floors, and walls. They have also been isolated from cardboard cages, floors, and walls of built rooms, which are called shelters of the chickens, by performing the next equation: Prevalence of shelter infestation (%): $(\text{no. of infested shelters} \times \text{total no. of shelters visited}) \times 100$. The regions from which soft ticks were collected were divided according to the directions of Mosul city: From the north: Saadah and Baawyza, Al-Arabi, and Al-Rashydiia (Blue dots). From east: Shuqaq Al-Khathrah, Al-Karamah, Gogjali, Al-Qadysia, and Al-Zahraah (Yellow dots). From the south: Al-Wahdaah, Somer, and Yaremjah (Red dots). Finally, from the west: Bab Al-Jadeed, Tal Alrumaan, Al-Yarmuk, and Hawii Alkaniisah (Green dots), as shown in figure 1. The collection period occurred between June 2023 and October 2023, and the numbers of males, females, and nymphs were collected each month of the research (14).

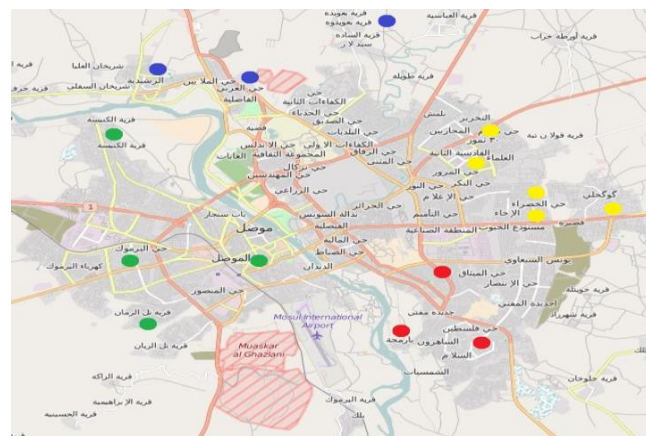


Figure 1: Showing the shelter areas in which samples of *A. persicus* are obtained.

Sample preparation

Samples have been collected and preserved in 70% ethyl alcohol; thereafter, they have been transported to the Parasitology Laboratory, College of Veterinary Medicine, University of Mosul. The samples have been allowed to dry on filter paper (15).

DNA extraction

Following the manufacturer's instructions, DNA was extracted from 32 ticks (larvae, nymphs, and adults) using AddPrep, a blood and tissue genomic DNA extraction MiniKit (AddBio, Korea). The extracted DNA has been used for isolating, identifying, and confirming the results of the concentration and purity of extracted DNA. Measurement of DNA concentration and purity.

Using NanoDrop (ThermoFisher, USA), the concentration and purity of DNA were evaluated. Pure DNA in the preparation is indicated by a ratio of 1.7 to 2.0 (16). The morality of DNA has been done by using gel

electrophoresis (14), and the DNA pellet has been rehydrated by adding 100 µl of rehydration solution (NanoDrop ND-100), and samples have been stored at -20°C until further assay (17). Polymerase Chain Reaction protocols have been used to amplify partial sequencing of mitochondrial genes, including 16SrRNA and COX1 (cytochrome C oxidase subunit 1).

Polymerase chain reaction procedure

PCR has been done using specific primers to confirm the molecular detection of all accrued *A. persicus* isolates 16SrRNA-F, 16SrRNA-R targeting the 16SrRNA gene, and COX1-F and COX1-R targeting the COX1 gene (Table 1). The primers were ordered from the Macrogen company in South Korea. The reaction of PCR mixtures has been prepared using AddBio MasterMix (2X) (AddBio, Korea). The cocktail mixtures of PCR have been processed in 20 µl containing a final concentration of 1X AddBio MasterMix, 1 µm of each primer, and 2 µl of DNA template (2 ng/µl). The PCR used a thermal cycler (T1100 Bio-Rad, USA). Table 2 clarifies the cycling conditions, including temperature and time.

Electrophoresis was done using 1.5% agarose gel to separate the amplified products. AddBio (Korea) was mixed

with 3 µl of Gel-Red dye (AddBio, Korea), and 5 µl of each PCR product was loaded into the well of the agarose gel. The electrophoresis was carried out at 75V for 1 hour using a 300-mA power supply and an electrophoresis tank (Bio-Rad, USA) containing 1X TBE buffer (GeNetBio, Korea). A 100 bp DNA marker, 6 µl (GeneDirex H3, Korea), was used as the standard molecular weight marker. The gel was examined under UV light using a gel documentation system (GelDoc EZ Imagine, Bio-Rad, USA) to document and determine expected bands (18). The PCR products have been run on ethidium bromide-stained agarose gels and observed by UV Trans-illumination (UVP BioDoc, Upland, CA, USA) (19).

Statistical analysis

The percentages of the data in this study were calculated, as it was confirmed that there were statistically significant differences using the Chi-Square test for independence. Suppose there were significant differences between the groups. The Bonferroni correction test was used to fix the locations of these differences, as all tests were conducted using (the IBM2 SPSS program) at a significant P-value, $P < 0.05$ (20).

Table 1: Sequences of primers used for amplification of specific genes of *Argas persicus*.

No.	Primer Name	Primer Sequence 5' – 3'	Product size (bp)	References
1.	16SrRNA-F	TTTGGGACAAGAAGACCCTATGAATTT	240	Zahid <i>et al.</i> (14)
2.	16SrRNA-R	ACATCGAGGTCGCAATCAATTTTATC		
3.	COX1-F	GGAGGATTTGGAAATTGATTAGTTCC	606	Yavari <i>et al.</i> (15)
4.	COX1-R	ACTGTAAATATATGATGAGCTCA		

Table 2: Cycling conditions of PCR for amplifying COX1 and 16SrRNA genes of *Argas persicus*

No.	Step	Temp °C	Time	Cycle
1.	Initial denaturing	95	10 min	1X
2.	Final denaturation	95	45 sec	35X
3.	Annealing	*	45 sec	
4.	Extension	72	1 min	
5.	Final Extension	72	5 min	1X
6.	Hold	4	4 °C	

*Annealing temperature was used for 16SrRNA gene= 56°C and COX1 gene = 53°C.

Results

Distribution and frequency according to the type of cages

An absolute of 764 ticks of *Argas persicus* have been collected from 390 domesticated chickens in 170 infested shelters with ticks. In distribution through shelters, wooden cages presence rate of 61.9%, cardboard cages 50%, and the existence rate was 0% in iron cages and 51.7% in built rooms (Figure 2) with significant differences in P value $P \leq 0.05$ depending on the type of the cages and statistically significant at $X^2 = 75.746$ in degrees of freedom = 4, So it has

been noticed that the higher rate of *A. persicus* presence was in wooden cages while absence of iron cages from ticks. The soft tick *A. persicus* distribution regarding all visited shelters was 36.47% (62/170).

Distribution and frequency according to the distribution of shelters in Mosul, city

The results of our work showed that the total infestation rate of *A. persicus* in chicken shelters in Mosul city was 36.47%. What did we mean by infestation of chicken shelters, the birds and their houses infestation, and the

chicken shelters infestation has been divided according to the four directions of the city, north, east, south, and west, so the highest prevalence of infestation rate was 11.76% which had been taken from east region of the city, while the lowest rate from the west region 6.47% with significant differences between two ratios as shown in figure 3 using equation aforementioned.

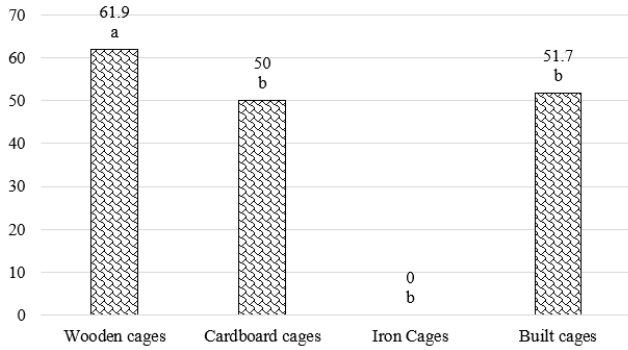


Figure 2: Distribution of *A. persicus* according to the types of chicken cages.

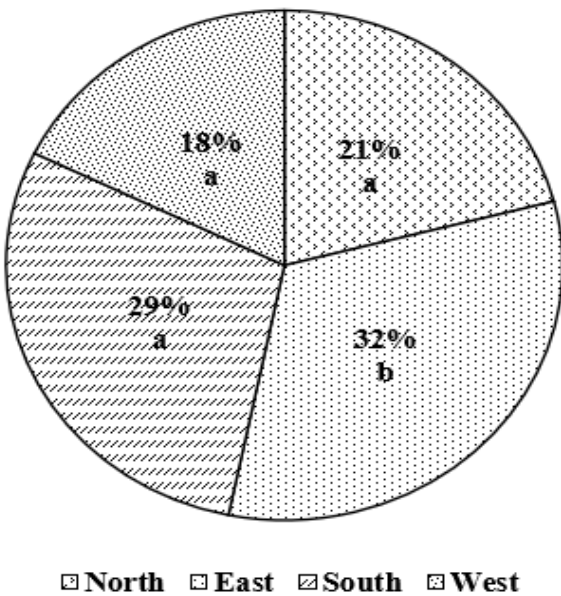


Figure 3: Prevalence and distribution of *A. persicus* infestation in chicken shelters in Mosul city.

Distribution and frequency according to months and seasons

The frequency of *A. persicus* infestation rates in the summer and fall seasons shows statistical differences at $X^2 = 8.244$ with degree of freedom =1 and P value = 0.004. The higher infestation rate is recorded in the summer 22.2% and 10% for the fall or autumn, as shown in figure 4.

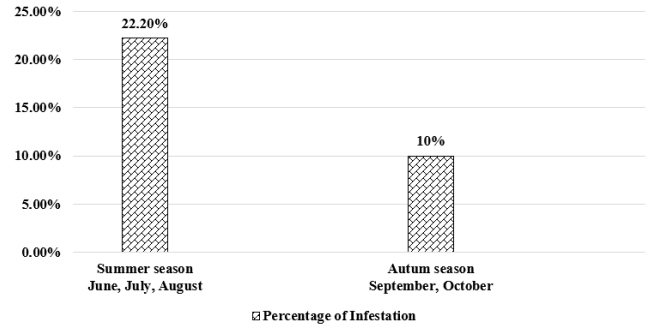


Figure 4: Frequency of *A. persicus* infestation in chickens during summer and fall seasons.

We reached a new result that explains the rising tick numbers through July in 33.4% frequency rate compared with those numbers and rates for other months, specifically (June, August, September, and October), as well as the significant differences in tick adult females among the months. The same applies to nymphs and larvae, while significant differences did not appear in male numbers between the months, and figure 5 explains these results.

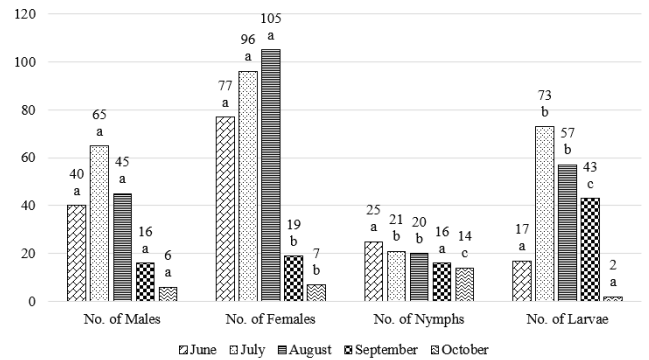


Figure 5: Frequency rates of different stages of *A. persicus* life cycle depending on the months of the study.

Molecular detection by PCR for the soft tick

Cocktail mixtures of PCR were processed in 20 µl containing a final concentration of 1X AddBio MasterMix. The range of DNA concentration readings was 34.1-66.7 ng/µl, with an average value of 45.15 ng/µl. The isolated DNA purity value ranged from 1.78 to 1.91, with an average purity value of about ~1.86. Regarding the COX1 and 16SrRNA specific primers, sequencing of the COX1 gene demonstrated that sixteen specimens in a previous study belonged to the soft tick *A. persicus* collections after DNA was extracted from *A. persicus* (Figure 6).

PCR intensification of each target gene of COX1 and 16SrRNA from singular DNA of *A. persicus* isolated proceeded in amplicons of the predicted size, which were 240 bp and 606 bp in length, respectively (Figures 7 and 8).

The thirty-two isolates showed effective PCR results following gel electrophoresis with 240 bp and 606 bp product sizes. Furthermore, all the isolates revealed positive outcomes for the COX1 and 16SrRNA genes.

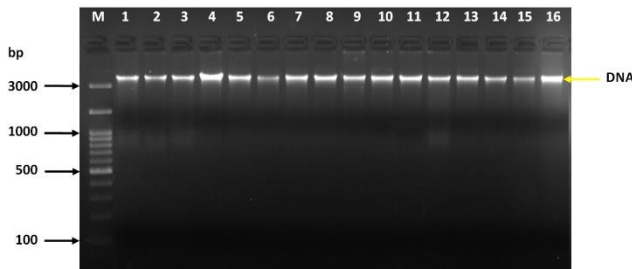


Figure 6: The result of agarose gel electrophoresis for extracted DNA from *A. persicus* well 1 represents (100 bp); DNA marker lanes 1-16 are positive DNA extracted specimens.

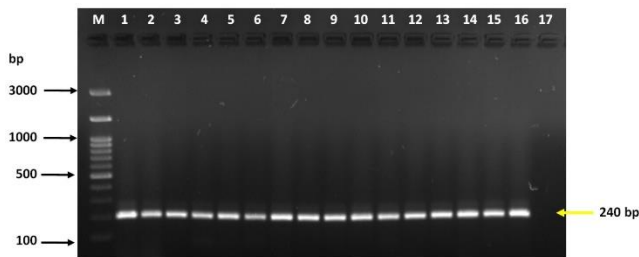


Figure 7: The result of PCR of 16SrRNA gene of *A. persicus*, lane M: (100 bp); DNA ladder. Lanes 1-16 are positive specimens, and lane 17 is negative control.

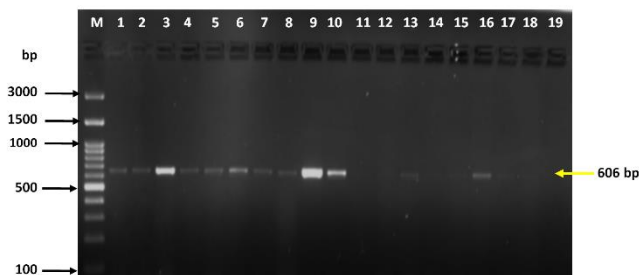


Figure 8: The result of PCR of COX1 gene of *A. persicus*. Lane M: (100 bp) DNA Ladder. Lines 1-10 and 13, 16 are effective specimens. Lane 11, 12, 14, 15, 17, 18 negative specimens, Lane 19 negative control.

Discussion

In previous study scrupulously classified soft ticks, *Argas persicus*, according to the taxonomic dichotomous key, which consists of many parameters. The ticks showed a

mamillated, granulated, written, tinkled, or striate structure with the advantage of having a pair of claws on each tarsus, which were carefully observed and documented in the research that *A. persicus* collected from local chicken and their shelters from many regions of Mosul city (north, east, west, and south of the city) (19-21).

The spread of ectoparasites has expanded, and they belong to many families, where they play a role as direct victors of abounding pathogens. The ectoparasites are the predominant discontent among humans and animals (22). Birds, including chickens, are affected by many pathogens on the feathers and other body parts of arthropods (23). The distribution of *A. persicus* fowl ticks through many shelters, like wooden cages, was the highest among other types of shelters, which agrees with the results (24,25). Our work showed that the total infestation rate of *A. persicus* in Mosul city reached 36.47%, and it came close to that obtained by Zahid *et al.* (14) 38.66% in Pakistan. At the same time, that outcome never agreed with Banafshi *et al.* (7) results for the rate of *A. persicus*, which did not exceed 8.02%. The reason for this is attributed to the unusual hosts besides the poultry that the ticks were collected from (cows, sheep, goats, lambs, turtles, poultry, and obscure hosts) in the borderline of Iran-Iraq in Kurdistan province, which placed in the west side of Iran (7). This research has explained the height of distribution rate of *A. persicus* on the eastern side of the city 11.76% compared to the rest of the areas, and this is due to the strategic condition of the eastern side of the city through the import traffic of animals (7). The study explained the contrast in frequency rates of *A. persicus* between the summer and fall seasons, and the reason is that the rate was higher in the summer followed by autumn in a decreasing trend; it was similar to that of Shahnaz *et al.* (26). distribution of ticks in any part of the world has been established, and these are related to several factors that service tick survival and growth, comprised of the topography of areas, rainfall sequence, relative humidity, aerial temperature, season, management practices, and husbandry. However, our results did not fit that of Lak *et al.* (27), who considered winter the most suitable season for *A. persicus* breeding. The differences in frequency of *A. persicus* might be due to the disparity in the landscape of the study region as well as climatic/environmental circumstances explained above related to the prevalence and frequency of ticks, and taking into consideration if the chicken may be exposed to diseases, also giving medications for affected birds (28,29). According to the results of this work, the number of ticks on top in July was higher than the others in the study, which is entirely consistent with the outcomes of Alzahrani and Edrees (9).

Regarding the nymphs and larvae of the soft-tick *A. persicus*, it had to be necessary to monitor their frequency rates during the study's months that the importance of different instars of ticks' life cycle in transmitting the pathogens (30-34). There has been a rise in the number of them throughout the summer months, as noticed by

Alzahrani and Edrees (9) and Mshelia *et al.* (35). The reason for this is attributed to the fact that the egg of *A. persicus* surface is coated with the wax lipids integrated by gene's organs, which are a complicated mix of alkalines as well as fatty acid esters in a long chain. These sheaths are a boundary conserving the embryo from accidental stresses and circumstantial factors and play a role as fungicidal agents, especially in high-temperature conditions (36-39). As for males of the ticks, there is no significant difference through the month's study. In our previous work, ticks have been analyzed morphologically. In the current one, samples have also been detected by mitochondrial ribosomal 16SrRNA and COX1 genes to verify the status of the taxonomy of *A. persicus*. Hence, molecular genetic interpretations are highly important in a place where 16SrRNA and COX1 genes were identified as applicable markers to consider their evolutionary properties. Following our work, we have found Crosbie *et al.* (40) and Chitimia *et al.* (41) advised that 16SrRNA arise as a suitable marker for phylogenetic tree analysis, also COX1 gene prone for this goal and matched with the similar other genes (15).

Conclusion

The present work is the earliest for recording the soft-tick *A. persicus* infesting local chicken in four sides of Mosul city, inclusive of (north, east, west, and south areas). The collection of ticks from their hosts and shelters was smaller in number and distribution in the western regions of the city, and it was concentrated in wooden cage shelters. The tick frequency for the chicken infestation in the summer months was higher than in autumn. The existence of soft ticks was validated at the molecular level by employing COX1 and 16SrRNA genes for the first time in Mosul and Iraq.

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Conflict of interest

The authors announced that there are no matches of concern about the publication of this manuscript.

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تكرار وتوزيع الخمج بالبرام الفارسي (عائلة القراد اللين) والتميز الجزيئي له في الدجاج المنزلي في مدينة الموصل

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الخلاصة

٧٦٤ قرادة من نوع البرام الفارسي جمعت من ٣٩٠ طائر من الدجاج المحلي من أربعة مواقع في مدينة الموصل. تم التقصي عن أماكن تواجد القراد في أقفاص الدجاج المختلفة، حيث كانت نسبة توزيع القراد في الملاجئ المصنوعة من الخشب هي الأعلى ٦١,٩%، ولم يكن هناك وجود للقراد في تلك المصنوعة من الحديد ٠,٠%، فضلاً عن ذلك فقد كانت نسبة الإصابة الكلية بالبرام الفارسي في ملاجئ الدجاج ٣٦,٤٧%، و أعلى نسبة الإصابة ظهرت في الملاجئ الواقعة شرقاً من المدينة ١١,٧٦% بينما أقل النسب فكانت تعود للمناطق الغربية من المدينة ٦,٤٧%، كما بينت الدراسة فروقات ذات دلالة إحصائية في نسب تكرار الإصابة بين فصلي الصيف والخريف ٢٢,٢ و ١٠% على التوالي، إن نسبة تكرار الإصابة بالقراد خلال تموز كانت الأعلى ٣٣,٤% مقارنة ببقية أشهر الفصلين ٢٠,٨، ٢٩,٧، ١٢,٤ و ٣,٧% كما كان بالإمكان ملاحظة الفروق المعنوية في أعداد ونسب إناث القراد البالغة خلال الأشهر ٢٥,٣، ٣١,٦، ٤,٥، ٦,٣، و ٢,٣% ويسري ذلك على الحوريات والبرقات بوجود الفوارق المعنوية في النسب والأعداد خلال أشهر الدراسة، وفيما يخص الذكور فلا وجود لفروقات ملحوظة بين أشهر الدراسة المختلفة. التحليل الجزيئي إلى ٣٢ عينة قراد، فتم عمل استخلاص دنا للقرادات المنفردة وعُرض لاختبار تفاعل السلسلة المتبلر بواسطة بادئات متخصصة لتضخيم القطعة من جين COX1 و16SrRNA وجميع اثنان وثلاثون عينة أظهرت نتائج فعالية لتفاعل السلسلة المتبلرة بعد الترحيل الكهربائي في الجل مع نواتج التفاعل بأحجام ٢٤٠ و ٦٠٦ زوجاً قاعدياً.