Research Article

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Morphological and Molecular Detection of Hard Ticks in Stray Cats in Mosul city, Iraq.

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Abstract

The results of the study showed the infestation rate of ticks in stray cats was 52.22% one type of hard tick was diagnosed *Rhipicephalus sanguineus* it was relied on the macroscopic examination and PCR technique was used for the existing species. The study recorded a high rate of infection in animals of age groups younger than one year with a significant difference depending on age and no significant difference was recorded in the infestation rate between males and female cats. The study also showed the spread of ticks in separate areas of the animal's body, where it was noted that the highest area of tick presence was in the ear and the back with an infection rate of 80.9% and 68.1%, respectively, while the lowest percentage of ticks was around the perianal and tail with an infection rate was 19.1%. The study also recorded a high rate of infection in the hot months with a significant difference in the percentage of infection depending on the months of the study

Keywards: Ectoparasite, *Rhipicephalus Sanguineus,* Stray Cat

الكشف عن القراد في القطط السائبة

الخلاصة

بينت الدراسة الحالية أن نسبة انتشار القراد في القطط السائبة في مدينة موصل بلغت 52.22%، وتم تشخيص نوع واحد من القراد الصلب *Rhipicephalus sanguineus* وتم الاعتماد في ذلك على الفحص العياني كما تم استخدام تقنية PCR للنوع المتواجد لتاكيد التشخيص. كما سجلت الدراسة ارتفاع نسبة الخمج في الحيوانات ذات الفئات العمرية الاقل من سنة مع تسجيل فارق معنوي بالاعتماد على العمر، في حين لم يسجل فرق معنوي في نسبه الخمج بين ذكور واناث القطط. كما بينت الدراسة انتشار القراد في مناطق متفرقة من جسم الحيوان حيث لوحظ أن أعلى منطقة لتواجد القراد كانت في الاذن وظهر الحيوان في حين كانت اقل نسبه لتواجد القراد حول الشرج والذيل. كما سجلت الدراسة ارتفاع نسبة الخمج في الاشهر الحارة مع وجود فرق معنوي في نسبة الخمج بالاعتماد على أشهر الدراسة.

Introduction

It is important in medical and veterinary care to study parasites in stray cats (1). These cats play a distinct role in the transmission of parasites between animals and humans as there are many common parasites between them (2) Therefore it is necessary to know and Identify the ticks that infect stray cats to develop methods of prevention and control to the human and animal health (3) Stray cats are infected with many of the parasites (4 ;5).Ticks are suck blood and obligate parasites cause skin disorders and direct problems to affected stray cats and cause stress, irritation, allergy ,weight loss and anemia (6), Ticks can transmit the widest range of bacterial, viral and protozoa diseases in humans and animals (7;8) The aim of the study is describing the infestion rate of ticks that feed on the stray cats by both morphology and molecular techniques because the macroscopic examination of ticks is not enough and PCR techniques is needed for species determination (9). This study the first report about the tick infestion of stray cats in Mosul city.

Materials and Methods

90 stray cats collected from different area of Mosul city – Iraq. The stray cats collected by catching them by making a trap by placing food (baited cage- Trap). All instructions and methods of animal welfare laws are used for the hunt animals in accordance with the Guidelines laid down by the International Animal Ethics Committee or Institutional ethics committee and in accordance with local laws and regulations (8). in all 57 female and 33 male cats were obtained each cat examined visually To mark the sex and age (by dentition) and grouped to 2 Categories less and equal than 1 year and more than 1 year and its location on the animals body all ticks were collected using non - toothed forceps ticks were examining under the dissecting microscope (10) Ticks put in ethanol alcohole 70 % and prepared for DNA extraction and molecular techniques using16SrDNA (11).

DNA Extraction of ticks:

DNA was extracted from 8 adult tick samples using gSYNC^{TM} Genomic DNA Purification kit (Genaid, Canada) with some modifications, as follows Ticks were incubated for 2-3 hours (depending on the size of insect) at 60 ºC in 300 µl of 200 µl of GST Buffer and 40 µl of Proteinase K solution, and 5µl RNase A Solution. After the initial digestion, adult ticks were homogenized utilizing first with sterile glass mortar then with glass mill. After homogenization, tubes were centrifuged at 10,000-×g to eliminate insoluble debris, while supernatants were collected and 200 µl of GSB Buffer then added and shaked vigorously for 10 seconds. 200 µl of absolute ethanol was added to the mixture and passed through provided column at 13,000 rpm for 30 seconds. Column was washed twice, one with 400 µl of W1 Buffer and with 600 µl of wash buffer, each step was followed by 14.000 rpm for 30 seconds. 100 µl of pre-heated Elution Buffer was added to the center of column then left for 5 minutes. DNA was collected in sterile 1.5 Eppendorf tubes by spinning at 14.000 rpm.

B- Polymerase chain reactions:

PCR techniques was used for confirmation the diagnosis of *Rhipicephalus sanguineus* using primers 16SrDNA forward 5′-TTGGGCAAGAAGACCCTATGAA-3′and reverse 5′-CCGGTCTGAACTCAGATCAAGT-3′ that amplify a 300bp to identification of *Rhipicephalus sanguineus*.The PCR was initial denaturation at 95cºfor 5 minute and then 10 cycles of 92cº for one minute annealing 48cº for one minute and extension at 72cº for 1.30 minute followed by a final extension at72 cºfor 7 minute (11; 12).

The PCR reaction mixtures

For each primers prepared in 30ml containing 15ml of mastermix, 1ml of the primers (forward & Revers) and 7ml of DNA template and 6ml of Distilled water .the PCR was used thermalcycler (T100, Bio Rad & USA) A 4ml of product loaded into well of agarose 1.5%gel. electrophoresis at 80V for 1 hour used power supply Mp 300 V (Bio - Rad , USA) containing 1 × TBE buffer (promega, USA) .A 300 bp DNA marker ,4ml marker the gel was examined under UV light using Gel doc Ez system (Bio – Rad, USA)(13).

Statistical analysis

Statistical analysis used Chi-square test by Jandel Sigma stat scientific softwareV3: one.

Results and Discussion

In the current study the 90 stray cats examined showed that 47 (52.2 %) were infected with one species of hard ticks *Rhipicephalus sanguineus*, This species collected were identified by morphology and molecular techniques fig (1,2,) respectively The current study registered the percentage of hard tick in stray cats in Mosul city was 52.22% which is different compared to other countries, in Dubai reported 4.2% (14) 80.1% in stray cats in Egypt (15) 0.8% in USA (16) 1.79 in Ireland (17) 52.4% in Hungary (18) and 1.6% in Brazil (19) the difference in the percentage of infection in countries may be related to seasonal & environments conditions, efficacy of control programs & different molecular Techniques help to identification of *Rhipicephalus sanguineness* because Identifications is not sufficient to detect the tick species (18).

Females were more infected than males were 56.1%, 45.5% respectively and No significant variation between male and female these results are agreement with those described by (20) who noticed that the male and females affected equally with the same factor of infection . The rate of infection was high in younger cats compared with older cats the statistical analysis was found that there is a significant variation between the ages (Table 1). The present study showed the infection rate was high in younger cats compared with older cats with significant difference was observed between the ages. Ticks of *Rhipicephalus sanguineus* were highly concentrated in different site on the body such as ears were (80.9%) Back and abdomen 68.1%, 63.8% respectively. The significant differences was found between the sites on the body (Table 2). . In this study *Rhipicephalus sanguineus* was more common in the ear and head and legs and

feet this study agrees with (21) who found that the most infected concentrated with *Rhipicephalus sanguineus* in the head , ear, legs and feet while the (22) who noticed that infection with *Rhipicephalus sanguineus* more Common attached between the toes.

Ticks of *Rhipicephalus sanguineus* were found in all months except January. The prevalence of Rhipicephalus *sanguineus* on stray cats ranged from (0 -90%) in January and both June and July (Table 3) ,Months dynamics is important factor for spread the hard tick of *Rhipicephalus sanguineus* in stray cats was much more prevalent in the hot months while the infection rate slowly in cold months this study this may be associated with the nature of ticks life cycle which is short in the hot months this results agrees with described by (23) who reported that the ectoparasites infection in stray cats was much more prevalent in Summer

Table (1) the infection rate of ticks of *Rhipicephalus sanguineus* according to the sex and age

Sex / Age	Male			Female		
	No.cats examined	No. cats infected	infection rate%	No.cats examined	No.cats infected	infection rate%
≤ 1 year	10	7	70 a	24	18	75 a
> 1 year	23	8	34.8b	33	14	42.4b
Total	33	15	45.5a	57	32	56.1a

Different letters in column differ significantly at level of $p<0.05$

Table (2) the infection rate of ticks of *Rhipicephalus sanguineus* according to the connected site on the body cats.

Connected site	No. of cats infected	Infection rate%
Head	22	46.8 a
Ears	38	80.9 b
Neck	8	17 c
Abdomen	30	63.8 a
Back	32	68.1 a
Legs & feet	11	23.4 c
Tail & perianal	9	19.1 c

Different letters in column differ significantly at level of $p<0.05$

Table (3) Prevalence of *Rhipicephalus sanguineus* in the cats according to months

Months	No. of infected	Prevalence %
December	2	20 a
January	0	0
February	1	10 a
March	4	40 b
April	6	60 b
May	8	80 c
June	9	90 c
July	9	90 c
August	8	80 c

The number of cats examined was 10 per months.

Different letters in column differ significantly at level of $p<0.05$

Figure 1: *Rhipicephalus sanguineus* male under the light microscope (4X)

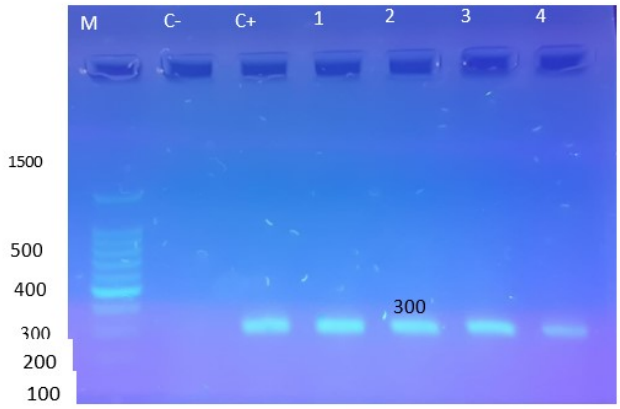

Fig (2) Gel electrophoresis by using 16S rDNA Showing M: size marker. Lane C+: positive control. Lane C -: negative control. Lanes 1-4: template DNA of *Rhipicephalus sanguineus*. At 300bp isolated from stray cats**.**

Conclusion

This study reports a high prevalence of *Rhipicephalus sanguineus* in stray cats Mosul city –Iraq.control of ticks in the stray cats remain agreat challenge so this study provides new information on infestation that may help in development control measure for cat's ectoparasites in Iraq.

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Conflict of Interest: The authors declare that there is no conflict of interest.

References

1-Beard D, Stannard H, Old M. Morphological identifcation of ticks and molecular detection of tick-borne pathogens from bare-nosed wombats (*Vombatus ursinus*).Parasit Vect. 2021;14 (60):5-11.

2-AL-Lahaibi BY, ALTaee AF..Detection of some species of lice and ticks infestation on local buffalo in Mosul city. Iraqi J of Vet Sci. 2018;3 (22):43-50.

3-Mustafa BHS. Detection on ectoparasites on small ruminants and their impact on the tanning industry in Sulaimani province. Iraqi J of Vet Sci. 2019;33(2):303-309.

4-Danielová V. Extension of *Ixodes ricinus* ticks and agents of tick-borne diseases to mountain areas in the Czech Republic.Int. J. Med. Micr. 2006;296 (2):48–53.

5- Taragelová VR,Mahríková L, Selyemová D, Václav R, Derdáková M. Natural foci of *Borrelia lusitaniae* in a mountain region of Central Europe. Ticks. Tick. Borne. Dis.2016; **7**(2): 350–356.

6-Stańczak J, Cieniuch S, Lass A, Biernat B, Racewicz M.Detection and quantifcation of

Anaplasma phagocytophilum and *Babesia* spp. in *Ixodes ricinus* ticks from urban and rural environment, northern Poland, by real-time polymerase chain reaction.Exp. Appl. Acarol. 2015;**66**(1):63–81.

7-Claerebout E.Ticks and associated pathogens collected from dogs and cats in Belgium. Parasit Vectors. 2013; **6**, 183.

8- Ebaniy V V, GuardoneL y, Marra F, Altomonte I.Arthropod-Borne Pathogens in Stray Cats from Northern Italy: ASerological and Molecular Survey. Animals. 2020;10 (2334).

9- Schreiber C. Pathogens in ticks collected from dogs in Berlin/Brandenburg, Germany. Parasit. Vectors. **Seven.**2014;(535):1-8.

10-Fourie J J, Joubert A, Labuschagné M, Beugnet F. New method using quantitative PCR to follow the tick blood meal and to assess the anti-feeding effect of topical acaricide against *Rhipicephalus sanguineus* on dogs. Comparative Immunology, Microbiology and Infectious Diseases. 2014;37:181–187.

11-Ghafar A, Khan A, Cabezas A, Gauci C, Niaz S.An Assessment of the Molecular Diversity of Ticks and Tick-Borne Microorganisms of Small Ruminants in Pakistan**.** Microorganisms .8: 2020;14-28

12- Faouzi M, Ahmed B, Saida M. Inventory of ticks on dogs in rural areas of the northeast of Algeria and its relationship with influences some ecological and climatic parameters. Iraqi J of Vet Sci. 2018;32(2): 175-182

13-Ismael SS, Omer LT.Molecular identification of new circulating *Hyalomma Asiaticum asiaticum* from sheep and goats in Duhok governorate, Iraq. Iraqi J of Vet Sci. 2021; 35(1): 79-83.

14-Schuster RK, Thomas K, Sivakumar S, Donovan D.The parasite fauna of stray domestic cats (Felis catus) in Dubai, United Arab Emirates. Parasit Res. 2009;105:125–134.

15-Aboelhadid S M,Waleed M,Arafa N..Molecular detection of *Rhipicephalus (Boophilus) annulatus* resistance against delta methrin in middle Egypt .Vet Parasit. 2018;13: 198-204.

16-Saleh M, Sundstrom K,Duncan K, Ientile M, Jordy J, Ghosh P. Show us your ticks: A survey of ticks infesting dogs and cats across the USA; Parasit Vect. 2019; 12(595):1-10.

17WaalT, LawlorA, Zintl A, Cowley BA .Survey of Ticks Infesting Dogs and Cats in Ireland. Animals (Basel). 2020; 10(1404).

18-Capari B, Hamel D, Visser M, winter R P,fister K, Rehbein S. Parasitic infections of domestic cats, felis cats, in western Hungary. Vet Parasit. 2013;192: 33–42.

19-Mendes D F, Crissiuma AL,Gershony LC,Willi L M, Paiva JP, Guerrero J, Labarthe N. Characterization of ectoparasites in an urban cat (Felis cats Linnaeus, 1758) population of Rio de Janeiro Brazil. Parasit Res. 2011;108(3): 1431–1435.

20- Kamaruddin NC, Adrus M, Wan WN. Prevalence of ectoparasites on a stray cat population from “Town of Knowledge” Kota Samarahan, Sarawak, Malaysian Borneo. Turk J

Vet Anim Sci. 2020;44:1-10.

21-Salant H, Mumcuoglu KY, Baneth G.Ectoparasites in urban stray cats in Jerusalem, Israel: differences in infestation patterns of fleas, ticks and permanent ectoparasites. Med and Vet Ento. 2013; 1-5.

22-Geurden T. Detection of tick-borne pathogens in ticks from dogs and cats in different European countries. Tick Borne.Dis. 2018;**9**(6): 1431–1436.

23-EL-Seify M A, Mohamed M G, Sultan K,Marey NM. Ectoparasites in stray cats in Alexandria province, Egypt: a survey study. Alexandria J of Vet Sci ·2016;48(1): 115-120.