Detection of *Dirofilaria immitis* antigen in cats in Mosul city

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**Abstract**

This study is the 1st trial to detect of *Dirofilaria immitis* in cats in Mosul city, Iraq. Dirofilariosis is the most dangerous nematodes which infect cats, through this study blood samples was collected from 200 cats (different ages, source and rearing management) to investigate from the mature female worms of *D. immitis* by using direct ELISA, results showed total percentage of infection was 7% (14/200 animals), the relationship between the infection with age of cats was proportional to the age of cats, as the percentage increases with the age of cats, the high prevalence of infection was recorded in cats more than 2 years old 4%, the kittens less than 1 year old showed low prevalence of infection 0.5%, statistically there is a significant differences between the oldest animals with other ages, significant differences between outdoor cats which recorded high prevalence of infection 4%, while Indoor animals recorded low prevalence of infection 3% with *D. immitis*, 10 of imported cat was infected with *D. immitis* with high prevalence of infection 5% when compare with native cats 2%, without significant differences between two groups, this study was conducted that the prevalence of *D. immitis* is high in adult cats, with high prevalence in outdoor rearing and the cats with imported origin.

**Introduction**

Several parasitic diseases affect cats (1,2) and the Dirofilariosis is the disease caused by nematode *Dirofilaria immitis* known as the heartworm because the site of the worms in the lungs arteries (right ventricle) (3), the disease distributed worldwide and affected both feline and canine species (4). Animals can be infected via female mosquito which can be faded to blood of infected animals, L3 can be developed from microfilariae in feline only (5). Vector adequacy effect of larval development of the mosquito individual (6). Then larvae transcend through the pharynx then gut which stay approximately 1 day (7). They then migrate to the Malpighian tubules and invade the cells then transformation to the so sausage stage L1 mute to the L2 stage then to L3 (8). L3 emerges from the folded labium and rests on the skin of the host immersed in a drop of haemolymph then enters the host (9). *Culex* and *Anopheles* is the main vectors of transmission of the disease the clinical signs of infected cats manifested as chronic (subclinical) or acute death form and mainly limited to respiratory signs and the disease called Heart Worm Associated Respiratory Disease (HARD) (10). The direct ELISA, is a perfect test to investigate protein of female worm’s genital tract the number of female heartworms in cats is so low of infection with male worms which high (11). Negative antigen test result in infected animals can caused by two reasons: immaturity female worms or decrease numbers of female worms, while false-positive results is low, so the antigen test is considered to screening test for heartworm infection in cats (12). Detection of *D. immitis* is attempt by using serological assays, thorax X-ray, and echo-cardiogram, microfilaria is present in circulating 6 months’ post infection, the serological assays are sensitive about 97%, they are considered to have good sensitivity, Therefore, the most effective methods for
diagnosis are chest X-ray, echo-cardiogram, and serological assays (13). Because there is no study which include the *D. immitis* in cats in Iraq, this study was aimed to detection of feline *D. immitis* antigens in cats using direct-ELISA.

**Materials and methods**

**Animals**

Two hundred of house hold and stray cats was examined. The age range of the cats was between 1 and 9 years old, estimated by the teeth, different source, rearing management.

**Blood collections**

A blood sample 2 ml was collected from cephalic vein of cat after sedation by the intramuscular injection of ketamine 7.5 mg/kg. The collected blood was added into tube without anticoagulant (11). Serum was separated from clotted blood in a non-additive tube (14).

**Direct ELISA**

Antigen from the mature female worms of *D. immitis* was detected by using an ELISA kit (DiroCHEK®, SYNBIOTICS Corporation, San Diego, CA 92127, USA). The ELISA procedures was attempt according the manufacturer’s manual.

**Statistical analysis**

The difference in the percentages of infection between the various clinical status and ages of cows were assessed by using two-sided Chi-square test in IBM-SPSS statistics version19 program (15).

**Results**

Table 1 recoded the prevalence of *D. immitis* antigen in cats with different age, the relationship between the infection with age of cats was proportional to the age of cats, as the percentage increases with the age of cats, the high prevalence of infection was recorded in cats more than 2 years old, the kittens less than 1-year-old showed low prevalence of infection, statically there is a significant difference between the oldest animals with other ages in significantly different (Table 1).

Outdoor cats recorded high prevalence of infection, while Indoor animals recorded low prevalence of infection with *D. immitis* with significant differences between it, in significantly different (Table 2).

Ten of imported cat was infected with *D. immitis* with high prevalence of infection when compare with native cats, without significant differences between two groups (Table 3).

**Table 1:** The relationship between the infection of *D. immitis* and age of cats

<table>
<thead>
<tr>
<th>Age</th>
<th>No. of cat examined</th>
<th>No. of positive(%)</th>
<th>(%) No. of Negative</th>
<th>P- Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Less than 1 year</td>
<td>67</td>
<td>1 (0.5)^a</td>
<td>66 (33)</td>
<td>0.07</td>
</tr>
<tr>
<td>≤1year-≥2years</td>
<td>61</td>
<td>5 (2.5)^a,b</td>
<td>56 (28)</td>
<td>0.5</td>
</tr>
<tr>
<td>≤2years</td>
<td>72</td>
<td>8 (4)^b</td>
<td>64 (32)</td>
<td>0.02</td>
</tr>
<tr>
<td>Total</td>
<td>200</td>
<td>14 (7)</td>
<td>186 (93)</td>
<td></td>
</tr>
</tbody>
</table>

Values significantly different at P<0.05 between cats different ages are labeled with the vertically different letters a, b, c.

**Table 2:** The relationship of percentage of infection of *D. immitis* with rearing condition of cats

<table>
<thead>
<tr>
<th>Rearing condition</th>
<th>No. of cat examined</th>
<th>No. of positive(%)</th>
<th>(%) No. of Negative</th>
<th>P- Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>House hold (Indoor)</td>
<td>154</td>
<td>6 (3)^a</td>
<td>148 (74)</td>
<td>0.001</td>
</tr>
<tr>
<td>stray (outdoor)</td>
<td>46</td>
<td>8 (4)^b</td>
<td>38 (19)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>200</td>
<td>14 (7)</td>
<td>186 (93)</td>
<td></td>
</tr>
</tbody>
</table>

Values significantly different at P<0.05 between cats different ages are labeled with the vertically different letters a, b, c.

**Table 3:** The prevalence of infection of *D. immitis* in Native and imported cats

<table>
<thead>
<tr>
<th>Source</th>
<th>No. of cat examined</th>
<th>No. of positive(%)</th>
<th>(%) No. of Negative</th>
<th>P- Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Imported</td>
<td>111</td>
<td>10 (5)^a</td>
<td>101 (50.5)</td>
<td>0.2</td>
</tr>
<tr>
<td>Native</td>
<td>89</td>
<td>4 (2)^a</td>
<td>85 (42.5)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>200</td>
<td>14 (7)</td>
<td>186 (93)</td>
<td></td>
</tr>
</tbody>
</table>

Values significantly different at P<0.05 between cats different ages are labeled with the vertically different letters a, b, c.
Discussion

*Dirofilaria immitis* is a widespread, infected parasite in cats, the parasite is not reported in cats in Iraq, so this study was reported total percentage of infection (7%), this percentage is so low, the reason of that may due to several causes spontaneous elimination of parasite, sudden death of animals, and the detection of microfilariar in peripheral blood is an unsatisfactory and microfilaria is rarely found (16). The results of the present study showed first occurrence of *D. immitis* in cats in Mosul city.

Several species of culicid act as vectors and play important role in *D. immitis* infection (17). Direct ELISA test give a positive result which indicated active adult worm infection and this result is highly specific, while the sensitivity is decrease when female worm two or fewer (18,19). The study conducted that the antibodies only indicate that an infection occurred and does not provide a guarantee that it still exists. There are variance results of the relationship between *D. immitis* infection and age of animals (20,21). The infection of *D. immitis* increase with increase of age because increase of duration of exposure to infection (vectors) (22). The results showed the infection in all age of animals and revealed the relationship of infection which affected according to health status, immune status and other infectious agents (23). Other reason is the longitude of duration of prepatent period of the *D. immitis* is 5-6 months (24). The results recorded differences between indoor and outdoor animals, other researchers approve this result of infection with *D. immitis* in dogs (25). The outdoors animals may increase the chance to exposure of vectors may be bearing infective L3 of *D. immitis* and live under bad conditions, those animals may have exposed to other infectious agents which may play important role in immunosuppression of outdoor animals (26), indoor animals or house hold animals Puddles a veterinary important care and owners care this decrease the chance of infection and live under quarantine condition this decrease exposed to vectors (24). Animals which imported from different countries reported high prevalence of infection when compare with native animals (27). *D. immitis* has been reported in some neighborhood countries otherwise countries from which animals are imported, Saudi Arabia (28), Romania (3), Iran (29), Russia (30), Croatia, Syria, Lebanon and Turkey (31), Egypt and Qatar (32), these researchers improve the importation of infected animals to transmission the infection from country to other country as emerging disease.

Conclusions

*D. immitis* was first investigated in cats in Mosul city, with a higher infection in <2 years old, imported, and stray cats.

Acknowledgment

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Conflict of interest

Author declare no conflict of interests of the manuscript.

References

النتيجة عن مستضد الخيطيات الدقيقة في القطط في مدينة الموصل

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الخلاصة

تعد هذه الدراسة من الأولى من نوعها للكشف عن طفيلي الخيطيات
الدقيقة في القطط في مدينة الموصل، بعد داش الخيطيات الدقيقة من
أخطر أنواع الديدان الإسفنجية التي تصب في الفم، حيث تم من خلال
هذه الدراسة جمع 200 عينة من قطر (مختلفة الأعمار والمباشرة)
فلاعة باستخدام اختبار المضاعن المائي المزود من النوع
المباشر، بنت النتائج ووجد نسبة إصابة كلية 7% (1400/1000)، وعند
إيجاد العلاقة فيما بين نسبة الإصابة وعمر الحيوان، تبين وجد علاقة
طرورية إذا ارتفعت نسبة الإصابة تقلد عمر الحيوان، حيث سجلت
القطط في التي تبلغ أكثر من 5 سنوات أكثر من معدل السالب 
حيث سجلت أقصى نسبة في القطط التي يبلغ
عمرها أقل من سنين 50% وترجع هذا بالطبع إلى افصاله الرغبة
الأكبر سنًا مع فن باقي الفئة العمرية. تبين أيضاً وجود فروق بين
القطط المراهنة، والتي سجلت أعلى نسبة إصابة 4% في حين سجلت
القطط المراهنة داخل الموصل أعلى نسبة إصابة 3.4%.

الخليصة

10 قطط مستورة أصابتها بالطفيليات الدقيقة إذا سجلت أعلى
نسبة إصابة % مقارنة بالقطط المحلية التي سجلت نسبة إصابة 3%،
ومع ذلك يوجد بعض منها أصابها، حيث سجلت نسبة
إصابة الخيطيات الدقيقة كان في القطط الأكثر سنًا وكان ذلك المراهنة.
خارج المنازل فضلا عن القطط المستورة.

14. Wasan AA. The serological diagnosis of canine Leishmaniasis by
114. DOI: 10.33899/j.vets.2019.163194

15. Leech NL, Barrett KC, Morgan GA. SPSS for Intermediate Statistics:
Use and Interpretation. USA: Lawrence Erbaum Assco; 2007.

16. Vieira L, Silvestre F, Fontes AP, Balreira AC. Seroprevalence of
heartworm (Dirofilaria immitis) in feline and canine hosts from
DOI: 10.1080/0022149X.2014.1000352

17. Walter T. Clinical aspects of dermatitis associated with
Dirofilaria repens in pets: A Review of 100 canine and 31 feline cases
(1990-2010) and a report of a new clinic case imported from Italy to Dubai.

18. Alahmed A, Al-Kurji MA, Kheir SM, Alahmedi SA, Hattabi MA,
AlGashmari MM. Mosquito fauna and seasonal activity in Makka Al
DOI: 10.1016/j.jksus.2010.12.001

19. Al-Kheiji AM. Survey and distribution of mosquito species (Diptera
culicidae) and description of its habitat in Riyadh district, Kingdom of
Saudi Arabia [master's thesis]. Riyadh: King Saud University Riyadh;
2005. [available at]

DOI: 10.1016/S0065-277X(08)00204-2

seroprevalence of Leishmania infantum infection in cats from northern
DOI: 10.1016/j.vetpar.2010.08.022

22. Song KH, Lee SE, Hayasaki M, Shirimizu K, Kim DH, Cho KW.
Seroprevalence of canine dirofilariosis in South Korea. Vet Parasitol.
2003;114:231-236. DOI: 10.1016/S0065-277X(03)00137-7

Seroepidemiologic survey of Dirofilaria immitis infection among
domestic dogs in Taipei city and mountain aboriginal districts in
10.1016/s0304-4017(01)00511-8

24. Rhee JK, Yang SS, Kim HC. Periodicity exhibited by Dirofilaria
immitis microfilariae identified in dogs of Korea. Korean J Parasitol.

25. Martin TE, Collins GH. Prevalence of Dirofilaria repens and
Dipetalonema reconditum in Greyhounds. Australian Vet J.

Occurrence of Dirofilaria immitis and tick-borne infections caused
by Anaplasma phagocytophilum, Borrelia burgdorferi sensu lato and
Ehrlichia canis in domestic dogs in France: Results of a countrywide
serologic survey. Parasitol Res. 2009;105:101-114. DOI:
10.1007/s00436-009-1501-2

27. Yildirim A, Ica A, Atalay O, Duzlu OA. Prevalence and
epidemiological aspects of Dirofilaria immitis in dogs from Kayseri
province, Turkey Res Vet Sci. 2007;82:58-63. DOI:
10.1016/j.rvsc.2006.08.006

of Dirofilaria immitis in dogs from the Hatay province, Turkey.

29. Omar IO, Elgailani A, Elamin SA, Omer AN. Serorelevance of
Dirofilaria immitis in dogs in Riyadh City, Saudi Arabia. Trop Biomed.
2018;35(2):531-540. [available at]

30. Tiatuna TV, Svetlana Y, Alexandru T, Heidrun T, Renke L, Egbert T.
Detection of Dirofilaria repens and Dirofilaria immitis DNA in
mosquitoes from Belarus. Parasitol Res. 2016;3(2):122-114. DOI:
10.1007/s00436-016-5188-y

(Dirofilaria immitis) and their vectors in Europe - new distribution