



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, Dirofilariasis 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.

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Introduction

Several parasitic diseases affect cats (1,2) and the Dirofilariasis 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 in to 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

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

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

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

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

Source	No. of cat examined	No. of positive(%)	(%)No. of Negative	P- Value
Imported	111	10(5) ^a	101(50.5)	0.2
Native	89	4(2) ^a	85(42.5)	
Total	200	14(7)	186(93)	

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

Discussion

D. immitis is wide spread 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 microfilaria in peripheral blood is un satisfactory 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 longtime 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 <2years old, imported, and stray cats.

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Conflict of interest

Author declare no conflict of interests of the manuscript.

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التقصي 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، يعد داء الخيطيات الدقيقة من أخطر أنواع الديدان الإسطوانية التي تصيب القطط، حيث تم من خلال هذه الدراسة جمع ٢٠٠ عينة دم من قطط (مختلفة الأعمار والمناشي فضلاً عن مختلف أساليب التربية) وذلك للكشف عن مستضدات الإنث البالغة باستخدام اختبار المتمز المناعي المرتبط الأيزيم من النوع المباشر. بينت النتائج وجود نسبة إصابة كلية ٧% (٢٠٠/١٤)، وعند إيجاد العلاقة ما بين نسبة الإصابة وعمر الحيوان تبين وجود علاقة طردية إذا ارتفعت نسبة الإصابة بتقدم عمر الحيوان، حيث سجلت القطط التي تبلغ أعمارها أكثر من سنتين أعلى نسبة إصابة بالخيطيات الدقيقة ٤%، في حين سجلت أقل نسبة إصابة في القطط التي يبلغ أعمارها أقل من سنة ٥,٥% وبفروق معنوية واضحة بين الفئة العمرية الأكبر سناً مع باقي الفئات العمرية. تبين إحصائياً وجود فروقاً بين القطط المرباة في خارج المنازل والتي سجلت أعلى نسبة إصابة ٤% في حين سجلت القطط المرباة داخل المنازل أقل نسبة إصابة ٣%، سجلت ١٠ قطط مستوردة إصابتها بالخيطيات الدقيقة إذ سجلت أعلى نسبة إصابة ٥% مقارنة بالقطط المحلية التي سجلت نسبة إصابة ٢% ومن غير وجود فروقاً معنوية إحصائية. بينت الدراسة أن أعلى نسبة إصابة بالخيطيات الدقيقة كانت في القطط الأكبر سناً وكذلك المرباة خارج المنازل فضلاً عن القطط المستوردة.