

Molecular detection of *Hammondia heydorni* in dogs in Mosul city

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Abstract

Through this study 122 individual fecal samples were collected from dogs, with different ages, sexes, and breeding management (stray and pet house hold). Some dogs were with healthy appearances while others were suffered from diarrhea, anorexia, depression, dehydration. Infection was investigated by light microscope after flotation technique by using Sheather's sugar solution. if the sample gives positive to oocyst (*H. heydorni* or other relative group), were mixed with potassium dichromate solution a rate of 2.5% for the stimulation of sporulation. DNA extraction was done using 2% sarcosyl, pronase E followed by phenol/chloroform extraction then precipitation attempt using ethanol. DNA amplification was attempt using *H. heydorni* primers JS4 and JS5 n microscope examination give 78 sample positives to the presence of oocyst, while polymerase chain reaction showed total percentage of infection with *H. heydorni* was 34.6% (27 case give positive reaction to PCR). Dogs less than 6 months of age show high infective rate 17.9%, dogs which suffer from gastrointestinal tract troubles (diarrhea, anorexia and dehydration) gave high infective rate 24.4%. Stray dogs gave high prevalence of infection to *H. heydorni* 25.6% when compared with pet house hold dogs which recorded 9% percentage of infection. This study is the first recorded *H. heydorni* in dogs in Mosul city.

Keywords: *H. heydorni*, Stray dogs, Pet dogs, Mosul

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الكشف الجزيئي عن طفيلي *Hammondia heydorni* في الكلاب في مدينة الموصل

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فرع الأحياء المجهرية، كلية الطب البيطري، جامعة الموصل، الموصل، العراق

الخلاصة

تم خلال هذه الدراسة جمع 122 عينة براز من الكلاب، حيث كانت بمختلف الأعمار والأجناس والسلالات (الكلاب السائبة والكلاب المنزلية) البعض منها كانت سليمة ظاهريا وبعضها الآخر كان يعاني من الإسهال، وفقدان الشهية والخمول والجفاف، تم التحري عن وجود أكياس البيض في براز الكلاب بواسطة الفحص المجهرى بعد إجراء تقنية التطويق باستخدام محلول شيدر، العينات الموجبة بأكياس البيض لطفيلي *H. heydorni* لتحفيز عملية التبوغ. تم استخلاص جزيئة الدنا للطفيلي باستخدام الساركوسيل 2% وإنزيم البرونيز ومن ثم الاستخلاص بواسطة الفينول / الكلورفورم ومن ثم الترسيب بواسطة الإيثانول، تم تضخيم جزيئة الدنا للطفيلي باستخدام البادئين JS4 و JS5 والمصممين من الجين ITS1. بينت نتائج الفحص المجهرى لأكياس البيض المتبوغة وجود 78 عينة موجبة بينما بينت نتائج تفاعل البلمرة المتسلسل لجزيئة الدنا للطفيلي وجود نسبة إصابة كلية بطفيلي *H. heydorni* 34,6% (27 حالة أعطت نتيجة إيجابية)، سجلت الكلاب التي بلغت أعمارها أقل من 6 أشهر اعلى نسبة إصابة 17,9%، في حين سجلت الكلاب التي تعاني من اضطرابات في الجهاز الهضمي (إسهال، خمول وجفاف) اعلى نسبة إصابة 24,4%. بينت النتائج أن اعلى نسبة إصابة بالطفيلي كانت في الكلاب السائبة 25,6% مقارنة بالكلاب المنزلية التي بلغت نسبة الإصابة فيها 9%. تعد هذه الدراسة هي الأولى في مدينة الموصل.

Introduction

H. heydorni is a coccidian obligatory parasite, the life cycle needs 2 hosts, the dogs and other wild candies is a definitive host, a numerous of herbivores is an intermediate host (1). Sexual reproduction and oocyst production occur in definitive hosts. The pre patent period is 1-3 weeks. The intermediate hosts can be infected by the ingestion of sporulated oocysts.

Definitive hosts become infected only after eating tissue cysts (2,3). Oocyst can excrete by definitive hosts and sporulated within 3 days (4). The infection with *H. heydorni* of the definitive hosts is characterized by subclinical course, some times the animals suffer from anorexia, severe diarrhea or recurrent diarrhea, unresponsive specially in puppies and immune compromised dogs, sometimes neurological signs have been observed (5).

Distinguishing of oocyst of the relative group of *H. heydorni* which include *Neospora caninum* and *Toxoplasma gondii* is so difficult (6,7), so that the differentiation between oocyst of these species using routine microscopic technique give inexact results (8). In order to detect the infection of *H. heydorni* a variable technique can be used which include serological and molecular technique (9,10), polymerase chain reaction is the best method for the detection and identification this coccidian parasite (11). The spacer internal transcribed spacer (ITS) can be used as genetic markers to detect this species of parasites (8).

This spacer is varied between *H. heydorni* and other coccidian parasites so this spacer can be used to discrimination between them (12).

Materials and methods

Fecal samples and morphological study of oocysts

This study was achieved in Mosul University, College of Veterinary Medicine, during the period from January 2019 to June 2019. A One-hundred and twenty individual fecal samples were collected from dogs with different ages, sexes, and breeds management (stray and house hold). Some animals healthy in their appearance and while others were suffering from diarrhea, anorexia, depression, dehydration.

The samples were examined to investigate the presence of oocysts by using light microscope after flotation technique was done using Sheather's sugar solution. if the sample gives positive to oocysts (*H. heydorni* relative group), were add to potassium dichromate solution a rate of 2.5% for the stimulation the sporulation in petri dishes at a room temperature for 10 days, sporulated oocyst were collected and purified by centrifugation and preserved at 4°C (8).

DNA extraction

A 0.5 ml of each sample was blended with 1.5mL of phosphate-buffered saline, then centrifuged at 14.000 g for 3 minutes. the Precipitate materials were washed 3 times in phosphate buffer saline and combined with 1.5 ml in micro tubes half hour. DNA was extracted with 2% sarcosyl (Merck), pronase E (Roche and with phenol/chloroform extraction then sedimentation attempt accomplished by using ethanol (13), DNA dissolved in RNA, DNA free water and preserve at -20°C.

DNA amplification

H. heydorni primers designed according to ITS1 sequences. The primers JS4 (5'-CGA AAT GGG AAG TTT TGT GAA C-3) and JS5 (5'-CAG CAG CTA CAT ACG TAG A-3) (Sigma Aldrich, Germany). reactions was done at 25 uL, the reaction include 25-100 pmol of two primers, dNTPs, buffer 1-2U Taq polymerase reaction mix contain 25-100 pmol of each primer, reaction buffer, dNTPs, and 0.5-2U of Taq DNA polymerase (D 1806 Sigma-Aldrich) and 1 ul of DNA sample (8), ten amplification attempt in thermocyclers at the program listed in table 1 were applied, size of the amplification products was 270 bp, then those products detected by using electrophoresis in a 2% agarose gel and ethidium bromide.

Table 1: Program of DNA amplification of *H. heydorni* using thermocycler

Step	Temperature (°C)	Time (minutes)	Cycles
Initial Denaturation	95°C	5	1
Denaturation	95°C	1	
Annealing	65°C	1	35
Extension	72°C	1.5	
Final Extension	72°C	10	1

Results

The results of sporulation and light microscope examination give 78 sample positive to the presence of oocyst, the DNA amplification were positive which revealed as band in 270 bp (Figure 1), the results showed total percentage of infection with *H. heydorni* is 34.6 % (27 case give positive reaction to PCR), animals less than 6 months of age recorded high infective rate 17.9 % while the lowest percentage of infection showed in animals more than 1 year of age (Table 2). The dogs which suffer from gastrointestinal tract troubles included diarrhea, anorexia and dehydration gives high infective rate 24.4% when compare with healthy dogs which showed 10.2% percentage of infection (Table 3).

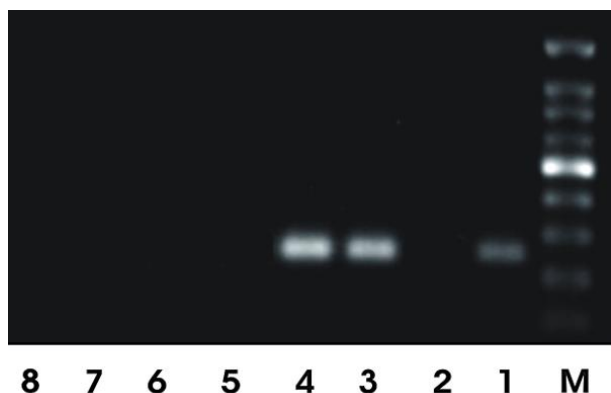


Figure 1: Result of electrophoresis of DNA products, M=marker, 1,3 and 4 positive samples, 2,5-8 negative samples

Table 2: Percentage of infection of *H. heydorni* in dogs using PCR according to age

	Age (months)			Total
	> 6	6 - 12	< 12	
Samples positive to <i>H. heydorni</i> or relative group n	33	26	19	78
Samples positive by PCR n (%)	14 (17.9)	8 (10.3)	5 (6.4)	27 (34.6)
Samples negative by PCR n (%)	19 (15)	18 (15.7)	14 (12.6)	51 (65.4)

Table 3: Percentage of infection of *H. heydorni* in dogs using PCR according to Health status

	Health status		Total
	Sick	Healthy	
Samples positive to <i>H. heydorni</i> or relative group n	47	31	78
Samples positive by PCR n (%)	19 (24.4)	8 (10.2)	27 (34.6)
Samples negative by PCR n (%)	28 (35.9)	23 (29.5)	51 (65.4)

Stray dogs give high prevalence of infections *H. heydorni* 25.6 % when compared with house hold dogs which recorded 9% percentage of infection (Table 4).

Discussion

There are no studies indicating the presence *H. heydorni* in dogs in Mosul city, so this study was done to determine the *H. heydorni* based on the detection of parasite DNA.

Table 4: Percentage of infection of *H. heydorni* in dogs using PCR according to Type of dog management

	Dog management type		Total
	Stray	House hold	
Samples positive to <i>H. heydorni</i> or relative group n	47	31	78
Samples positive by PCR n (%)	20 (25.6)	7 (9)	27 (34.6)
Samples negative by PCR n (%)	27 (34.6)	24 (30.8)	51 (65.4)

This study showed total percentage of infection with *H. heydorni* is 34.6 % (27 case give positive reaction to PCR), and high prevalence of infection in dogs less than 6 months and lowest percentage showed in more than 1 year of age. Few studies dole out the relationship between the prevalence of infection of *H. heydorni* and the age of dogs, (14) recorded high prevalence of infection to *H. heydorni* in dogs 3-6 months of age, while (15) reported the prevalence of infection of *H. heydorni* was 2.7% from 481 fecal sample of dogs, Muhammed (16) reported high prevalence of infection in dogs 1-4 years old and lower percentage in older dogs, (17) reported affected dogs are generally less than six months old, the reasons of increase the percentage of infection younger animals is the nature infection of *H. heydorni* in the definitive hosts which take subclinical form specially in younger and immunosuppressed animals (5), younger dogs immune system can exposed to several etiological microorganisms which cause immune suppression which lead to allow *H. heydorni* to invade gastrointestinal tracts infection and appearing clinical signs.

High infective rate in animals suffer from gastrointestinal tract troubles, Abel (1) report that the infection of *H. heydorni* is increasingly becoming associated with diarrhea and gastrointestinal tract problem, Webb (18) found the oocyst of *H. heydorni* in feces of a dog suffer from intermittent bouts diarrhea this dog already been treated with immunosuppressive treatment. Stray dogs give higher prevalence of infection with *H. heydorni* when compared with pet house hold dogs, the reason of that is due to periodic vaccination program and preventive medicine to another parasitic disease in pet housed dogs, which lead to decrease the possibility of immune suppression, another reason is the use of commercial dog food to feeding the pet dogs while stray dogs are free feeding (19). In contrast to the stray dogs, pet dog health care is crucial in terms of veterinary follow-up with periodic antiparasitic treatment (20).

The role of veterinarian visits has an important role in the reduction of disease occurrence. Our results confirm this hypothesis, since recurrent visits to the veterinary

clinics by pet dog owners has a positive impact on the health of their animals.

Conclusions

Hammondia heydorni was affected dogs more than 1 year of age and in dogs suffer from GIT troubles and this parasite was first recorded in dogs in Mosul city.

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Conflict of interest

Authors declare no conflict of interests of the manuscript.

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