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 الجزائري عن طفيلي

في الكلاب في مدينة الموصل

 ومن أمجد العبدي

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 الساندة والكلاب المنزلية) البعض منها كانت سليمة ظاهريا وبعضها الآخر كان يعاني من الإسهال، وقد قام الكهف والخلاف، تم التحري عن وجود أكياس البيض في براز الكلاب بواسطة الفحص المجهرى بعد إجراء تقنية التطويل باستخدام محصل شير. العينات الموجبة بآكاس البيض لطفيلي للحياض عامل التبوع. تم استخلاص جزيئة الدنا للطفيلي باستخدام الساركوسيل 2% وازيم البروتين ومن ثم الاستخلاص بواسطة الفينيل / الكرورقوم ومن ثم الترسيب بواسطة الإيثانول. تم تشخيص جزيئة الدنا للطفيلي باستخدام اليدتين JS4 و JS5 والمصممين من الجين ITS1 والبلمرة المتسلسل لجزيئة الدنا للطفيلي ووجد نسبة إصابة كلية بطفل في *H. heydorni* 34.6% (77 حالة أعطت نتيجة إيجابية)، سجلت الكلاب التي بلغت أعمارها أقل من 6 أشهر أعلى نسبة إصابة 17.9%, في حين سجلت الكلاب التي تعاني من اضطرابات في الجهاز الهضمي (إسهال، خمول وجفاف) أعلى نسبة إصابة 24.4% بينما النتائج أن أعلى نسبة إصابة بـ*H. heydorni* كانت في الكلاب الساندة. 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 (ºC) | Time (minutes) | Cycles |
|-----------------------|------------------|----------------|--------|
| Initial Denaturation  | 95ºC             | 5              | 1      |
| Denaturation          | 95ºC             | 1              |        |
| Annealing             | 65ºC             | 1              | 35     |
| Extension             | 72ºC             | 1.5            |        |
| Final Extension       | 72ºC             | 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 |
|--------------|-------|
| H. heydorni or relative group n | 78 |
| Samples positive | 27 |
| by PCR n (%) | (34.6) |
| Samples negative | 51 |
| by PCR n (%) | (65.4) |

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

| Health status | Total |
|---------------|-------|
| H. heydorni or relative group n | 78 |
| Samples positive | 27 |
| by PCR n (%) | (34.6) |
| Samples negative | 51 |
| by PCR n (%) | (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.

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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