Molecular study of *Cryptosporidium* spp. and *Giardia lamblia* which cause diarrhea in camels (*Camillus dromedaries*) in Al-Diwaniyah and Al-Najaf provinces /Iraq

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Abstract

Because of little studies which related with camels this is the first study in Iraq aimed to description of *Cryptosporidium* spp., and *Giardia lamblia* in it. Out of 200 fecal samples of camels from the slaughter house and other areas of Al-Diwaniyah and Al-Najaf provinces. Some of camels suffered from diarrhea and the others asymptomatic animals within the period from October 2015 to the end of April 2016. The study showed the prevalence of *Cryptosporidium* infection was (55%) by Modified Ziehl-Neelsen stain method, while the prevalence of *Giardia* infection was (20%) by Lugol's iodine and by floatation with zinc sulfate solution. Regarding to the prevalence depending the PCR technique there were (88%) of *Cryptosporidium* infection while the prevalence of *Giardia* infection was (39%). This study concluded that the camels consider source of infection with both genus of studied parasites which consider zoonotic parasites. The routine examinations necessary to detection the zoonotic diseases.

Key words: *Cryptosporidium* spp., *Giardia lamblia*, camels, modified Ziehl-Nelseen stain, PCR technique.

دراسة جزيئية لطفيلي الأبواغ الخبيئة وطفيلي الجيارديا المسببة للإسهال في الجمال لمحافظتي الديوانية والنجف / العراق

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الخلاصة

لقلة الدراسات التي تتعلق بالجمال في العراق هدفت الدراسة التحري عن طفيلي الأبواغ الخبيئة وطفيلي الجيارديا فيها وقد تضمنت الدراسة فحص (200) عينة براز للجمال من مختلف مناطق محافظتي الديوانية والنجف وكذلك من مجزرتي المحافظتين خلال الفقرة من تشرين الأول 2015 ولغاية نيسان عام 2016 والتي كان قسم منها تعاني من إسهال شديد والقسم الآخر لا تعاني من اي اعراض سريرية ظاهرية .أظهرت نتائج الدراسة الحالية مدى انتشار طفيلي الأبواغ الخبيئة وطفيلي الجيارديا حيث كانت نسبة الاصابة بطفيلي الأبواغ الخبيئة الكلية (بنسبة 55%) باستخدام طريقة صبغة زيل نلسن المحورة بينما كانت نسبة الاصابة بطفيلي الجيارديا (20%).اما فيما يخص تقنية تفاعل السلسلة المتبلمرة PCR فكانت نسبة الاصابة بطفيلي الابواغ الخبيئة (88%) بينما كانت نسبة الاصابة بطفيلي الجيارديا (39%) .استنتجت الدراسة ان الجمال تعتبر مصدر للإصابة بكلا الطفيليين موضوع الراسة اللذان يعتبران من الطفيليات المشتركة ، الفحوصات الدورية ضرورية لتحديد الامراض المشتركة.

الكلمات المفتاحية: انواع طفيلي الابواغ الخبيئة *Cryptosporidium* spp. ، طفيلي الجيارديا *Giardia lamblia* ، الجمال ، صبغة زيل نلسن المحورة ، تقنية تفاعل السلسلة المتبلمرة PCR.

Introduction

Cryptosporidiosis is one of the important zoonotic diseases, the causative agent is parasite known as *Cryptosporidium* and the fecal-oral route is the mode of transmission of this disease, it infects many vertebrates, involving human beings (1). The recent

review of (2) summarizes in detail the molecular components and mechanisms involved in the development life cycle of *Cryptosporidium.* Molecular characterization of camel *Cryptosporidium* revealed that *C. parvum, C. muris* and *C. andersoni* can infect camels, *Cryptosporidium parvum* infection in young camels can lead to severe diarrhea, emaciation, dehydration and death (3,4,5). In Egypt were reported Cryptosporidia from camels (*Camelus dromedarius*) without identification of the species. Detection of *Cryptosporidium* oocysts by microscopy most commonly is done by direct smear and without any staining and by the modified Ziehl-Neelsen stain under light microscopy, immunofluorescent antibody-based (IFA) procedures have a high sensitivity, but still the easier and cheaper traditional staining methods such as the Ziehl-Neelsen stain are widely used, despite their lower sensitivity (6). The common methods of detection of *Cryptosporidium* spp. in feces were enzyme-linked immunosorbent assay (ELISA), microscopy and/or real-time PCR (7). *Giardia lamblia* (Syn; *Giardia duodenalis, Giardia intestinalis*) is the causative agent of giardiasis, a gastrointestinal infection of humans, companion animals, livestock and wild life. Signs of a *Giardia* infection vary from asymptomatic to profuse diarrhea as well as chronic disease (8). Diagnosis involves testing for *Giardia* trophozoites and cysts in direct unstained fecal smears to look for motile trophozoites or using Lugol's iodine to help distinguish the cysts and trophozoites (9)**.** A trichrome stain of saved stool is other method used to describe *Giardia* (10). The study aims to detection of protozoa causative agent *Cryptosporidium* spp. and *Giardia lamblia* cause diarrhea in camels by microscopic examination and by PCR.

Materials and methods

1-Fecal samples collection:

Total of 200 fecal samples were collected from camels, (some of this camels was suffered from diarrhea and others were asymptomatic camels) in the slaughter house and other areas in Al-Diwaniyah and Al-Najaf provinces during the period from October 2015 to the end of April 2016. Samples were collected in a sterile plastic containers and stored in the large containers containing ice bags, then transported to the parasitology laboratory in Al-Qadisiyah University to perform the examinations.

2-Microscopic examination:

-The direct smear method by modified Zeihl -Neelsen stain: making light smear and staining with modified Zeihl - Neelsen stain (11)

-The direct smear method by Lougal Iodine and floatation method with zinc sulfate solution: according to (12)

3-Polymerase chain reaction (PCR):

The PCR technique was performed for detection *Cryptosporidium* spp. based subunit ribosomal rRNA gene from camel stool samples. This method was carried out according to method described by (13) as following steps:

A-Genomic DNA Extraction: Genomic DNA from camel feces samples were extracted by using AccuPrep® stool DNA Extraction Kit, Bioneer. Korea, and done according to company instructions.

B-Genomic DNA Profile: The extracted DNA was checked by using Nanodrop spectrophotometer (THERMO. USA), which measured DNA concentration (ng/µL) and check the DNA purity by reading the absorbance at (260 /280 nm).

C-PCR Master Mix preparation: PCR master mix was prepared by using (AccuPower® PreMix Kit) and this master mix done according to company instructions.

D-PCR Thermocycler Conditions: All PCR reactions were done at same thermocycler conditions by using convential PCR thermocycler system.

E-PCR product analysis: The PCR products was analyzed by agarose gel electrophoresis.

Results

The prevalence of *Cryptosporidium* infection according to microscopical examinations, was 55% (110/200 fecal samples) in Al-Diwaniyah and Al-Najaf

provinces (Fig. 1). The prevalence of *Giardia* infection (Fig. 2) according to microscopical examination was 20% (40/200 examined fecal samples). The prevalence of *Cryptosporidium* infection according to polymerase chain reaction (PCR) technique was (88%) (88/100 fecal sample examined) (Fig. 3).The prevalence of *Giardia* infection according to PCR technique was 39% (39/100 examined fecal samples) (Fig. 4).

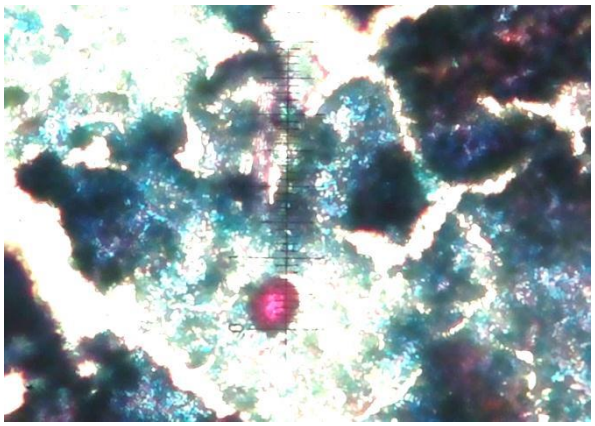

Fig. (1): *Cryptosporidium* spp. oocysts in camel feces, using Modified Ziehl-Neelsen stain(X100).

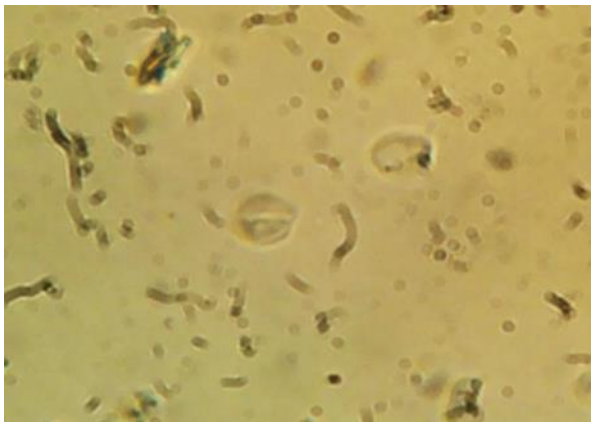

Fig. (2): *Giardia lamblia* trophozoite in camel feces shows the exon, (Floatation method with Zinc Sulfate solution)(X100).

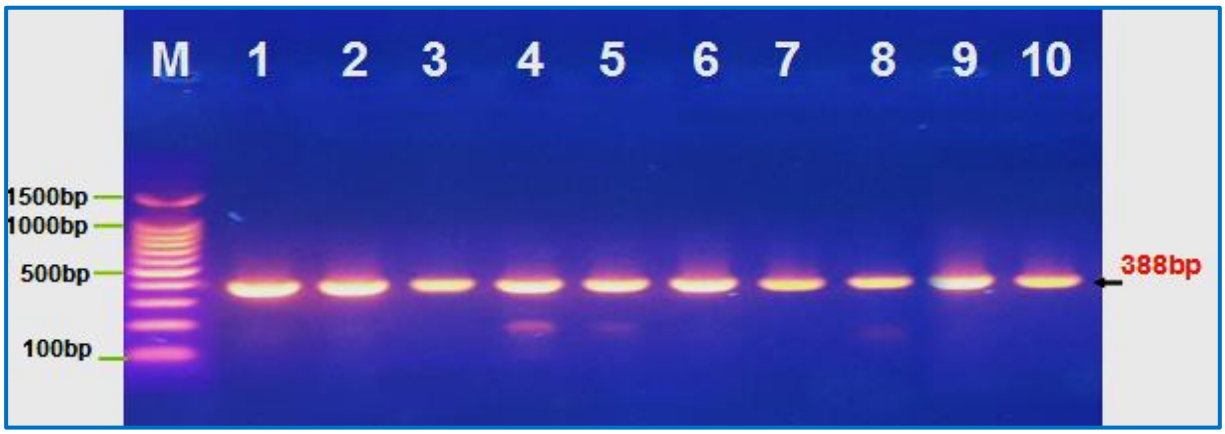

Fig. (3): Agarose gel electrophoresis image that show PCR product analysis of small subunit ribosomal RNA gene in *Cryptosporidium* spp. from camel feces samples. Where M: Marker (1500-100bp) and lane (1-10) some positive samples for *Cryptosporidium* spp. at 388bp product size.

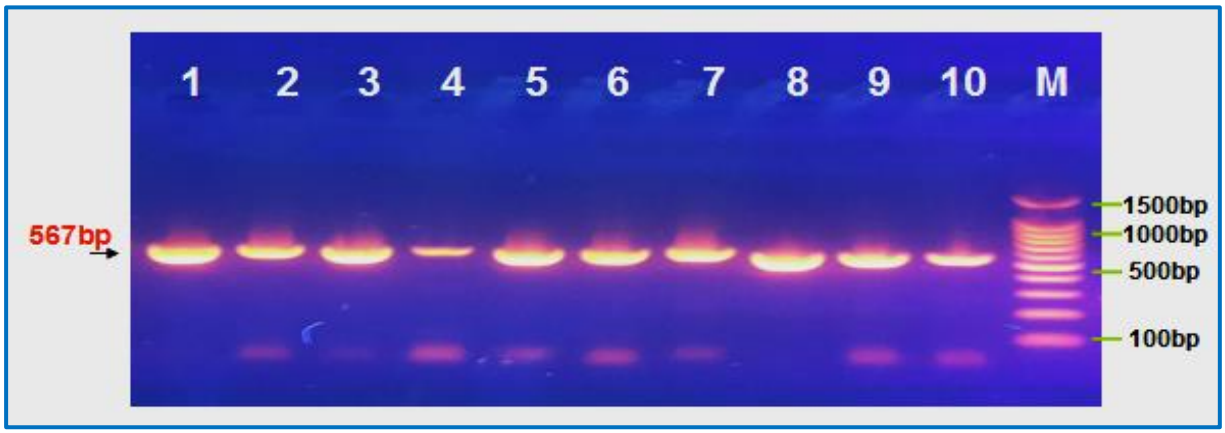

Fig. (4): Agarose gel electrophoresis image that show PCR product analysis of small subunit ribosomal RNA gene in *Giardia lamblia* from camel feces samples. Where M: Marker (1500-100bp) and lane (1-10) some positive samples for *Giardia lamblia* at 567bp product size.

Discussion

The prevalence reported by microscopic diagnosis according to Modified Ziehl-Neelsen stain under light microscopy in camel is (55 %). This result was agreed with (14) in Al-Najaf province who reported 60% rate of *Cryptosporidium* infection in camels by microscopic examination with Modified Ziehl-Neelsen stain. Also it is agreed with (15) in Najaf-Abad in Isfahan province, Iran, which observed *Cryptosporidium* oocyst in 39 out of 103 camels (37.9) %. But disagreed with the result of (16) in Yazd Province, Iran which reported the prevalence 20.33% , (17) in Iran and (18) in Qeshm Island, South of Iran when they reported 16.9% and 1.9% respectively and at (19) were observed 10% of examined camels in Iran. Also (20) observed 4.7% in Kerman, Iran. (4) noted that *Cryptosporidium* infection rate in camel in Egypt 17.5% and 19.3% respectively. Also (21) observed the prevalence of *Cryptosporidium* infection was 22.3% (55/246) in cattle of northwest Iran. (22) observed prevalence rate of *Cryptosporidium* infection in domestic and wild animals were 6% in domestic and wild animals in central California coast. While (23) in Portugal and (24) in Tunisia found that, no positive cases were detected in examined camels. The differentiation in results may due to the number of camels examined and the climate, time and the environmental condition. The prevalence *Cryptosporidium* infection reported by PCR technique in camel in both Al-Diwaniyah and Al-Najaf provinces (88%). This disagree with the prevalence rate reported by (24) in China (20.3%) was detected in 12/352 (3.4%) samples tested by PCR in horses in the Czech Republic and Poland (26). Also disagree with results of (27) in giant panda in China which reported 17% (17/100) adult pandas were identified as cryptosporidium positive by PCR. The prevalence of *Giardia* infection reported by microscopic diagnosis according to normal saline 10% and lougal iodine in camel (20%). These results congruence with the result of (10) which was reported the prevalence rate of *Giardia* infection 24% in camels according to microscopic examination. Also agreed with the result of (28) in Baghdad which reported the prevalence 14.6% in cattle. And conforms with (29) in Al-Diwaniyah province which reported 21.41% in cattle and (30) were they showed prevalence (29%) in captive mammals at the zoo of Zagreb, Croatia. (31) in Peshawar, Pakistan were reported 24 positive samples of 150 fecal samples (16%) in ruminants. (32) in Baghdad when he reported 26.4% in cattle too. (33) in ThiQar which was observed prevalence 12.9% in cattle. Also (34) observed prevalence rate of *Giardia* infection in domestic and wild animals were 15%. While disagreeing with study occurred in Australian calves the prevalence rate were 58% (35). In USA the prevalence in cattle 52% (36). In Canada other studies reported that the prevalence rate of giardiasis in calves was 70% (37). In Iran the prevalence rate was 37.6% in cattle also (38). Because of little studies about this parasite in camels we compared with some ruminants to detect the prevalence of Giardia in animals. These differences between the prevalence of giardiasis in the present study and prevalence of other studies in the other regions and countries may be related to many factors including environmental changes, number of samples were collected, study season, laboratory methods which used in diagnosis, in addition, the experience of examiner all these factors affect in the final image to infection of *Giardia* (39). The prevalence *Giardia* infection reported by PCR technique in camel in both Al-Diwaniyah and Al-Najaf provinces (39%). This agrees with fingers of (40) in Western Australia which found that the prevalence of positive *Giardia* by using PCR was 44% in sheep, and agreed with results of (41) in Karbala province which reported the prevalence rate 45.7%, 40% and 53.3% in cattle, sheep and goat respectively. But disagree with study in Iran, prevalence rates was 15.9% of *Giardia duodenalis* by PCR in sheep and goat in Ahvaz (42). PCR-based prevalences were 10.9% for *G. duodenalis* in southern Ethiopia (43). Small amount of samples used for examination which may not contain the parasite due to scanty infection (44).

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