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Prevalence Rate of Isolated*Cryptosporidium* Spp. ofBroiler Chickenin Al-QadisiyahProvince

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Abstract
This study has been carried out to determined the prevalence rate of cryptosporidiosis in broiler flocks. 150 fecal samples from 30 broiler flocks (5 samples for each flock) in different areas of AL-Qadisiyah province were used. They were collected starting of January till November 2016 with two age groups of chickens, (10-15 and 30-35) days old. Samples were examined with Modified Ziel -Neelsen stain to detect the positive samples by microscopic examination. After that, a nested-PCR technique was performed on 60 samples. Results of microscopic examination showed that total infection rate of *Cryptosporidium* was29.33% . The highest rates of infection were recorded in 10-15 days group and during spring season, infection rates reached were 35.71% and 34.28% respectively. The lowest rates were observed in 30-35 days group and during summer season were 23.75% and 20% respectively. No significant differences within percentage in both two age group or among percentage in seasonsat level ($P < 0.05$) were recorded. According to Nested Polymerase Chain Reaction test, the results showed that overall percentage of infection is20%. The highest rate of observed infection is 23.33% in 10-15 days aged group, while the lowest rate of infection is (16.67 %) in the other age. No significant differences was observed between percentage in ages. On the other hand, result of comparing nested-PCR test with microscopic examination shows that there are no significant differences between the percentage of both tests.
In conclusion, cryptosporidiosis is widespread in broiler flocks in AL-Qadisiyah province, but there is no significant impact found concerning the relationship between infection rates and age of chickens or seasons of the year.

Introduction

Cryptosporidiosis is one of the essential protozoan infections in birds.It affects a major number of avian species across various continents(1). It iscausing either clinical or subclinical infections (2). There are three forms of Avian Cryptosporidiosis: respiratory form, intestinal form and renal form (3).Clinical signs of a respiratory form include cough,nasal discharge, sneezing, dyspnea pneumonia and thickening of air sacs(3). Clinical signs of enteritis form include yellow greenish diarrhea, offensive odor, depression, low feed consumption and high water consumption(4).Transmission of *Cryptosporidium*parasiteis through ingestion of oocysts of the infected individuals by contaminated water and/or food(5, 6).The parasite is in Phylum Apicomplexa and part of the group of parasites commonly referred to as Coccidia, which includes*cryptosporidium*,*Eimeria*,*Cyclospora* and *Isospora*(7).*Cryptosporidium*infectionrepresents the main public health concern of water utilities in developed nations(8).
Identified species of *Cryptosporidium*in birds are *Cryptosporidium parvum*,*Cryptosporidiumbaileyi*,*Cryptosporidium Meleagridis*and*Cryptosporidiumgalli*(9).
The prevalence rate in broiler chickens varies in different countries. In Iraq,20.71% existed in Karbala(10).In Iran, infection rate is 23.8% (11). In Henan, Chinaprevalence rate

was3.4%(12).In Tunisia, the prevalent rate is4.5% (13).In Greece, *Cryptosporidium* oocysts were found in 24.2% of the examined broiler chickens(14).

Due to the importance of the cryptosporidiosis on the health of poultry, this study is designed to detect the parasite in the broilerflocksof AL-Qadisiyah provinceand study the effect of ages andseasons on the prevalence of cryptosporidiosis.

Materials and Methods

Feces samples collection: 150 Fecal samples from 30 broiler chickens flocks (5 samples for each flock)in some regions ofAL-Qadisiyahprovincewere used.They have been collected from the beginning of January until November 2016 with two age groups (10-15 and 30-35) days old broiler chickens, the study includesfour seasons (60 days for each season) in which they are divided as shown intable (2). The fecal sample has been transferred to a clean, dry plastic container and transported to the laboratory for the analysis.

Microscopic examination the oocyst is detected by examining each sample by pigmented the swab by Modified Ziel -Neelsen (MZN)(15).Subsequently a Nested polymerase chain reaction testhas been performed on 60 samples. The data have been analyzed by SPSS program, using Chi-square test(X^2).

DNA Extraction

DNA has been extracted from feces samples by using (Stool DNA extraction Kit, Bioneer. Korea). The extraction has been done according to company directives by using stool lysis protocol method with Proteinase K. Subsequently, the extracted DNA has been checked by NanoDrop spectrophotometer, Thence stored (-20C) at refrigerator until used in PCR amplification.

Nested Polymerase chain reaction

PCR techniquehas been performed for diagnostic of *Cryptosporidium*parasite. based on 18S rRNA gene by using specific primers are designed by (16). the first round primers forward primer (GACATATCATTCAAGTTTCTGACC) and reverse primer (CTG AAGGAGTAAGGAACAACC) has been amplified (763bp) product size and the nested primers forward primer (CCTATCAGCTTTAGACGGTAGG) and reverse primer (TCTAAGAATTTCACCTCTGACTG)has been amplified (587bp) product size . These primers are provided by (Korea: Bioneer company). The PCR positive samples of first roundhave been used in nested amplification at the same amplification condition to amplified (587bp) product size.

Result and Discussion

Diagnostic characterization of *cryptosporidium spp* of microscopically examinationbyusing Modified Ziel -Neelsen stain when examined under high oil emersion (100) lens of microscopic as in figure (1)showsoval-shaped or spherical objects with a color red or dark pink on a blue ground.

Table (1) shows no significant differences in infection rates between age of chickens (10-15 and 30-35) days old.The results show that the highest rate of infection (35.71%) that is observed in the ages 10-15 days, While the lowest rate (23.75%) is in 30-35 days . This is consistent with the results of (12)which are observed in broiler chickens aged from 1 to 20 days have the highest rate of infection more than from 21 to 60 days. But these resultsdo not agree with what (10), who found thatthe highest rate of infection at the age of five weeks (28.72%) and the lowest at the age of two weeks (13.18%).However, the minimum prevalence ratewas in the younger age group(11).

Table (2) shows that there are no significant differences among the seasons.the highest rate (34.28%) is seen in spring ,While the lowest rate (20%) is in summer and (32.5) (31.42) are seen in winter, autumn respectively. This is partly in line with what (12) have foundspring season is the highest rate of infection in chickens and decreases significantly in autumn and summer seasons. Whilewinter season is the lowest rate of infection.

Table (3) reveal the results of nested-PCR as showed in figure (2) .The results show that the highest rate of infection (23.33%) is observed in the age group of10-15 days, while the lowest rate (16.67%) is in 30-35 days of agewith no significant differences between the percentages in both twoage group at level ($P < 0.05$).

The results of examination show that among (150) samplesexamined microscopically,

44(29.33%) have given positive.While the totalinfection rate was 20% (12/60)in the nested-PCR testand no significant differences between the percentages in both tests at level ($P < 0.05$) Table (4).

The different prevalence rates in broiler chickens between different studies such as3.4% in broilers (12).The overall infection rate of *Cryptosporidium* was 10% (17).The percentage of infection is 20.71%(10).Total infection rate is 23.75%(11).The difference in prevalence rates observed may due to the animal management differences (18). On the other hand, the use of different diagnostic methods may also be responsible.

The result of the comparison betweenNested PCR and Microscopic Examinationin this study is partly in line with what (19) which have shown that the infection rate of microscopic examinationis51%in sheep. Then all positive checked by Nested- PCR examination, The results show that 19 (37.3%) sheep samples out of 51 casesare positive.

Table (1) Microscopic Examination of Broiler Chickens According to the Age

Age groups	Examination No	Positive No	Percentage %
10-15 days	70	25	35.71 A
30-35 days	80	19	23.75 A
Total	150	44	29.33

Non – significant differences at $P < 0.05$ due to X^2 tab. = 3.84146 > X^2cul. = 1.40297.

Table (2) Microscopic Examination of Broiler Chickens According to the Four Seasons (Two months for each season)

Season	Examination No	Positive No	Percentage %
Winter(Jan. and Feb.)	40	13	32.50 A
Spring (21 Mar. to 20 May)	35	12	34.28 A
Summer (July and Aug.)	40	8	20 A
Autumn (20 Sept. to 20 Nov.	35	11	31.42 A

Non – significant differences at $P < 0.05$ due to X^2 tab. = 7.81473 > X^2cul. = 3.22527.

Table (3) *Cryptosporidium*According to Nested Polymerase Chain Reaction

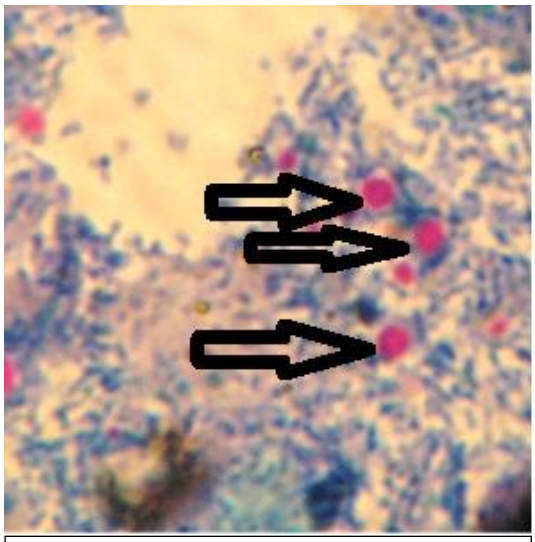
Age groups	Examination No	Positive No	Percentage %
10-15 days	30	7	23.33 A
30-35 days	30	5	16.67 A
Total	60	12	20

Non – significant differences at $P < 0.05$ due to X^2 tab. = 3.84146 > X^2cul. = 0.27799.

Table (4) A Comparison between Nested Polymerase Chain Reaction and Microscopic Examination to Diagnose*Cryptosporidium*

Total	Examination No	Positive No	Percentage %
Total ME	150	44	29.33 A
Total NPCR	60	12	20 A

Non – significant differences at $P < 0.05$ due to X^2 tab. = 3.84146 > X^2cul. = 1.14261.



Figure(1) :-shows *cryptosporidium* stained with Modified Ziel -Neelsen stain magnification (100x)

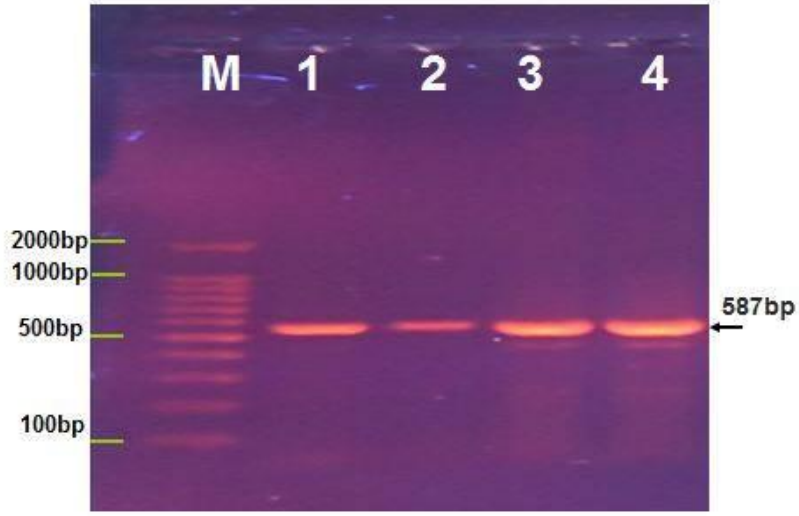


Figure (2)Agarose gel electrophoresis picture that shows the Nested PCR product of 18S rRNA gene used in the detection of*Cryptosporidium* spp of broiler chicken fecal samples. Where M: Marker (2000-100bp), lane (1-4) positive of *Cryptosporidium* spp at 587bp PCR product size.

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معدل انتشارداء الابواغ الخبيئه المعزولة من افراخ دجاج اللحم في محافظة القادسية

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الخلاصة

هدف الدراسة الحالية معرفة نسبة الاصابة لداء الخبيئات في حقول دجاج اللحم. جمعت 150 عينة لبراز دجاج من 30 حقل (5 عينة لكل حقل) من مناطق مختلفة في محافظة القادسية خلال الفترة الممتدة من شهر كانون الثاني الى نهاية شهر تشرين الثاني 2016 وكان عمر الدجاج في تلك الحقول بعمري (10-15 و 30-35) يوم. كل العينات فحصت باستخدام المجهر الضوئي بعد التصبيغ بصبغة زيل نلسن المحورة لتحديد العينات الموجبة. بعد ذلك اختبرت 60 عينة بتفاعل السلسلة المتبلمرة المتداخل - nested).(PCR

نتائج الفحص المجهري اظهرت ب أن معدل الاصابة الكلية لداء الخبيئات كان 29.33% وكانت اعلى نسب للإصابة (35.71) (34.28) بعمر 10-15 يوم وموسم الربيع على التوالي بينما كانت اقل نسبة للإصابة (23.75) (20) بعمر 30-35 يوم وموسم الصيف على التوالي. ولم يكن هناك فروق معنوية بمستوى (P <0.05) عند المقارنة بين عمري ومواسم التجربة

نتائج الفحص الجزيئي اظهرت بأن معدل الاصابة الكلية كانت بنسبة20% ولم يكن هناك فروق معنوية بمستوى (P <0.05) عند المقارنة بين عمري التجربة حيث كانت اعلى نسب الاصابة (23.33) بعمر 10-15 يوم بينما كانت اقل نسبة اصابة (16.67) بعمر 30-35 يوم.

من جهة اخرى اظهرت نتائج التجربة بين الفحصين (الفحص المجهري، تفاعل السلسلة المتبلمرة المتداخل) عدم وجود فروق معنوية بين نسب الاصابة لكلا الفحصين.

نستنتج من خلال نتائج هذه الدراسة ان الاصابة بداء الخبيئات واسعة الانتشار في حقول محافظة القادسية غير ان ليس لعمر الدجاج او مواسم السنة تأثير معنوي على نسبة الاصابة.

الكلمات الافتتاحية :داء الابواغ الخبيئة ، دجاج اللحم، معدل الاصابة ، الفحص المجهري،تفاعل السلسلة المتبلمرة.