Using species-specific PCR technique to detect *Toxoplasma gondii* in broiler chickens

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Abstract

Two groups of broiler chickens were used in this study. One was reared under typical conditions at the animal house of Veterinary Medicine College/Mosul University- Iraq, while the other group was reared under common commercial farm conditions. Fifty and 80 birds from the two respective groups were sacrificed at 49 days of age for detecting *Toxoplasma gondii* by using Species-specific PCR technique. Results of Latex agglutination test indicated, principally, that 29.3% and 49.2% of the serum samples were positive for the birds of both groups, respectively. Titer figures ranged between 1:20 to 1:320 where the highest value was 1:160 (39.3%) and the lowest was 1:20 (5.8%). Confirmation of 38 and 64 serum samples, using Latex agglutination test was performed by PCR technique, from the two respective groups of chickens. Of those, 8 samples from the college birds and 35 from the commercial farm birds were confirmed positive by giving band of 133 bp, according to specific primers designated on gene B1.Based on these results, pursuing the PCR technique is considered, so far, a most sensitive method for *Toxoplasma gondii* detection. Also, positive PCR results are counted on as an early marker for reactivation and useful means in monitoring therapies.

Keywords: *Toxoplasma gondii*; Broilers; PCR; Rearing conditions.
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استخدام تقنية تفاعل البلمرة المتسلسل للنوع المتخصص للكشف عن المقوسة الكوندية في فروج اللحم

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الخلاصة

استخدمت في هذه الدراسة مجموعتين من فروج اللحم بعمر ٤٩ يوم للكشف عن المقوسة الكوندية باستخدام تقنية تفاعل البلمرة المتسلسل، تم ذبح ٥٠ دجاجة كانت مرباة تحت ظروف تربية مثالية في بيت الحيوانات التابع لكلية الطب البيطري في جامعة الموصل بينما ذبحت ٨٠ دجاجة كمجموعة ثانية مرباة في حقول التربية التجارية. أظهرت النتائج أولا أن ٢٩,٣% و ٤٩,٢% كانت موجبة لاختبار تلازن اللاتكس في المجموعتين على التوالي. معايير الأضداد تراوحت بين ١:٢٠ و ١:٣٢٠ وكانت أعلى قيمة (٣٩,٣%) عند المعيار ١:١٦٠ وأقل قيمة (٥,٨%) عند المعيار ١:٢٠. تم تأكيد النتائج الموجبة (٣٨ و ٦٤) لكلا المجموعتين من الدجاج في اختبار تلازن اللاتكس بواسطة تقنية تفاعل البلمرة المتسلسل، كانت ثمانية عينات من فروج الكلية و ٣٥ عينة من دجاج الحقول موجبة بتفاعل البلمرة المتسلسل من خلال ظهور حزمة بوزن جزيئي ١٣٣ زوج قاعدي اعتمادا على البادئ الخاص المصمم على جين ب ١. اعتمادا على النتائج المستحصلة من تقنية تفاعل البلمرة المتسلسل لهذا تعتبر هذه الطريقة حساسة في الكشف عن المقوسة الكوندية.

Introduction

Toxoplasmosis is a worldwide zoonosis of increasing concern in both human and veterinary medicine (1). The major source of *Toxoplasma* infection for susceptible animals is *Toxoplasma* oocysts excreted in cat feces (2). Toxoplasmosis which is caused by the obligate intracellular protozoan *Toxoplasma gondii*, is responsible for major

economic loss in all classes of livestock through abortions, stillbirth and neonatal losses (3,4). Epidemiological studies in Europe have indicated that meat consumption could account for an almost 60% of the *Toxoplasma* infections, whereas contact with soil (gardening) may be held responsible for approximately 20% of the cases, according to Cook *et al*., (5). Chickens are also a potential source of *Toxoplasma* infection in humans according to Tenter *et al.,* (6). On the other hand, the parasites are transmitted directly through ingestion of infective oocysts in food or indirectly, via consumption of the undercooked meat of farm animals who had ingested the oocysts. Fetus can be infected via transplacental route from a mother who has been infected during pregnancy (7).

Early in 1963, Siim *et al*., (8) reported that 63 species of birds became infected from ingestion of oocysts and developed cysts in their tissues without passing oocysts to their fecal materials. As far as poultry houses are concerned in the matter of toxoplasmosis, it has been found by (9-11) that rats and mice are victims of scattered cats in surrounding areas of poultry houses which may be considered as a main source for spreading *Toxoplasma gondii* oocysts. Insects like cockroaches and earth worm are also considered an important reservoir for *T. gondii* oocysts (12). Thereby, *Toxoplasma gondii* is a ubiquitous intracellular protozoan parasites that chronically infect approximately one-third of the adult human population in the United States and up to 85% in parts of Europe (6). Approximately 85% of women at childbearing age in the United States are susceptible to acute infection with the protozoan parasite *T. gondii* (13). Dubey (14) isolated *T. gondii* at a percentage of 39% from chickens under extensive and free living management. Aspinall (15) revealed the presence of the parasites in 38% of the commercial meat retail outlets in UK. Others, Dubey *et al.,* (16) reported the existence of 10.4% isolates of *T. gondii* in free-range chickens in Austria. Mahmood *et al.,* (17) have reported that 81.81% of the 220 broiler serum samples were positive for *T. gondii,* by latex test. Similar study was performed by (18) indicated that a random samples of broiler slaughterhouse showed 63.3% of 150 serum broiler samples were positive for *T. gondii* by using latex test, in Nineveh governorate, Iraq.

As for the diagnosis of *T. gondii,* the traditional procedure requires the detection of the tachyzoites of *T. gondii* in histologic section of the brain. However, brain biopsy is considered too aggressive measure for routine use (19). During the last two decades, polymerase chain reaction (PCR) has been widely used for detection of *T. gondii* in clinical samples from patients with toxoplasmosis (20). Most investigators have used the *B1* gene for detection of *T. gondii* in various biological specimens (21-23).

Due to the fact that 39.33% of pregnant women in Mosul city - Iraq, are infected with *T. gondii*, according to (24), the aim of this study is to elucidate the role of broilers as one of intermediate hosts that play a role in maintenance of infection in the environment,through the detection of *Toxoplasma gondii* antibodies in broiler chickens and confirming the infection by using PCR technique.

Materials and methods

Blood samples

One hundred and thirty broiler chickens used in this study. Two blood samples from each individual bird was obtained (50 samples from those reared under typical management conditions, at animal house of veterinary medicine college, and 80 samples from broilers reared under normal commercial farm conditions). The collected blood samples from each bird were kept in a clean and sterilized test tubes without anticoagulant for serum collection (plane tube) and with anticoagulant for DNA extraction. Clotted blood samples were kept in a refrigerator overnight. Serum was separated by centrifugation at 3000 rpm for 15 minutes and stored at – 20 °C till examination.

Another non-clotted 130 blood samples were stored at – 20 °C till DNA extraction and amplificated by using polymerase chain reaction technique.

Latex agglutination test (LAT)

A semi quantitative latex agglutination test was done according to the manufacture instructions using Toxocell-latex, Bio kit com., Spin.Briefly, 50 µl of normal saline were added to the antigen containing plate from section 2 through 6 (Index 1). Fifty µl of serum samples were placed onto side section 1 and 2 of test plate. By mixing the sample and saline solution on section 2 several times till they were well mixed. Thereafter, 50 µl of the made mixture on section 2 transferred to section 3. Mixing the reagents was done by repeating the aforementioned process through section 6, thereafter 50 µl of diluted mix were discarded, according to (25).

Index 1: Illustration of serum dilution procedure.

Section	1	2	3	4	5	6
Saline µl	.	50	50	50	50	50
Serum µl	50	50	.	.	.	.
Mix and transfer µl	50	50	50	50	50	50
Dilution	1:1	1:2	1:4	1:8	1:16	1:32
IU/ml	10	20	40	80	160	320

DNA isolation and PCR amplification of Toxoplasma

The isolation of DNA from blood samples of both groups of chickens was secured, using a commercial purification system, (wizard Genomic DNA purification Kit; Promega, Madison, W1). For amplification of *Toxoplasma gondii*, PCR assay was used according to (26). The primers used in present study as an essential PCR elements were designed on B1 gene (133 bp), where the foreword 5′ TTG CAT AGG TTG CAG TCA CT 3′ and the reverse is 5′ TCT TTA AAG CGT TCG TGG TC 3′,according to Van de Ven *et al.,* (20). The amplification reaction mixture (final volume, 25 µl) contained 10 mM Tris-HCl (pH 8), 50 mM KCl, 1.5 mM $MgCl_2$, deoxynucleoside triphosphate at a concentration of 200 µM, 10 pmol primer, 1 U of *Taq* DNA polymerase, and 100 ng of genomic DNA. Amplifications were carried out with thermal cycler. The programme of amplification reaction consisted of one denaturation step at 94°C for 2 min, followed by 40 cycles consisting of denaturation at 94°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 45 s. A final elongation step at 72°C for 5 min was performed. Amplification products were visualized in a 1.2% agarose, using gel electrophoresis stained with ethidium bromide. A Ladder 100 bp DNA was used as a size marker in the gels.

Results and discussion

Results of this study proved the occurrence of considerable percentages of *T. gondii* infection in broiler chickens. However, the percentage of *T. gondii* occurrence was substantially less among birds reared under well sanitized poultry houses such as the one used in study. Table 1 shows the positive results of *Toxoplasma gondii* by latex agglutination test for the two groups of tested chickens from which blood samples were obtained, with 64 samples (49.2%) from the chickens reared under normal commercial farm conditions and 38 samples (29.3%) from animal house at the College of Veterinary Medicine. These results agree with the findings of (9). Titer of the positive samples were ranged from 1:20 to 1:320. The highest number of positive samples were from birds of the commercial farm was 28 with a titer 1:160 (27.55%) while those obtained from the animal house were 12 (11.80%), with above mentioned titer. The number of positive samples with a titer of 1:80 was found to be 16 (15.7%) and 14 (13.7%), respectively. On the other hand, the number of samples having a titer of 1:40 was 9 (8.8%) and 5 (4.9%), respectively. The number of positive samples with a titer of 1:320 was 7 (6.9%) and 5 (4.9%), respectively. Finally, positives with titer 1:20 were found to be 4 (3.9%) and 2 (1.9%), for the two groups of chickens, respectively.

Table 1: *Toxoplasma gondii* antibody titers and their percentages in tested blood samples.

Source		Commercial farm		Animal house		Total	
		No.	%	No.	%	No.	%
Antibodies Titers	1/20	4	3.9	2	1.9	6	5.8
	1/40	9	8.8	5	4.9	14	13.7
	1/80	16	15.7	14	13.7	30	29.4
	1/160	28	27.5	12	11.8	40	39.3
	1/320	7	6.9	5	4.9	12	11.8
No. of Samples		80		50		130	
No. of +ve samples		64 (49.2%)		38 (29.3%)		102 (78.5%)	

Table 2 reveals the confirmed positive samples to latex agglutination test by PCR technique. The 38 positive blood samples by LAT test collected from the animal house showed only 8 positive samples by PCR technique which represented 6.2%, whereas, the 64 positive blood samples by LAT test from commercial farm showed only 35 samples being positive by PCR technique, (26.9%), while Al-Sanjary (18) in his study confirmed the positive samples to latex agglutination test by polymerase chain reaction techniques, of 95 positive blood samples by LAT test collected from the two poultry slaughterhouses, 52 samples were positive by PCR technique with a percentage of 34.7%.

Table 2: Confirmation of PCR results for positive Latex agglutination in tested blood samples.

Source	Commercial farm		Animal house		Total	
	No.	%	No.	%	No.	%
No. of +ve samples in Latex test	64	49.2	38	29.3	102	78.5
No. of +ve samples in PCR test	35	26.9	8	6.2	43	33.1
No. of Samples	80		50		130	

Figure 1 represents PCR amplification of 43 *Toxoplasma gondii* DNA isolates using specific primer (133 bp). It is evident from figure 1 that 1,2,4,5 lanes represented the positive samples while lane 3 represent the negative sample. Lane M indicates the Ladder 100 bp DNA marker.

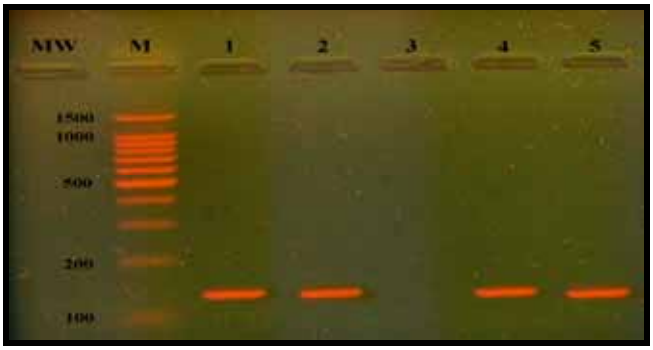

Figure 1: Represents PCR positives to *Toxoplasma gondii* of broiler blood, using specific primer (133 bp*).* Lanes 1,2,4,5 represent positive samples and lane 3 indicates negative. Lane M indicates the Ladder 100 bp DNA marker.

These results implied a public health significance, especially through consumption of undercooked poultry meat. It can be emphasized on that PCR technique is considered a sensitive tool for the diagnosis of toxoplasmosis where a positive results can be counted on as an early marker for reactivations. Also, this technique proved to be a powerful tool for monitoring therapy.

According to the results of this study, a poultry manager should endeavor to make the best of a very difficult situation for sake of securing typical conditions in rearing broiler chickens.

Conclusion

Results of detecting *Toxoplasma gondii* by PCR technique for two groups of chickens revealed that this type of parasite is easily spread around unhygienic poultry houses,causing the broiler carcasses to be carrier for this parasite.

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