Determination of *Toxoplasma gondii* lineages of sheep in Wasit, Iraq

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Abstract

Toxoplasma gondii is an intracellular parasite that can cause significant morbidity in human beings and animals. Up to our knowledge no data is known of genetic diversity of *T. gondii* in sheep in Iraq. This study aim to detect the strains (genotypes) of *T. gondii* isolates from sheep in Wasit province, east of Iraq. A total of 315 samples (blood 300 and placenta's tissue 15) were collected from aborted ewes, which initially had been examined serologically by LAT, then further tested by RT-PCR through *B1* gene amplification to confirm the infection with *T. gondii*. After that, the positive DNA samples were assayed for genetic characterization depending upon nested PCR- RFLP of *SAG2* gene. Out of 315 examined samples, 10 were confirmed positive *T. gondii* DNA. The genotyping assay of them revealed that 60% (6/10), 30% (3/10) and 10% (1/10) of examined isolates represent the genotypes of II, III and I respectively. The type II appeared as dominant in sheep in Wasit province, Iraq.

Keywords: Toxoplasmosis, Genotype, Ovine, Iraq
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تحديد أنساب مقوسة كوندي في الأغنام في واسط، العراق

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الخلاصة

مقوسة كوندي هو طفيلي يعيش داخل الخلايا والذي يمكن أن يسبب اصابات مرضية كبيرة في البشر والحيوانات. وحسب معلوماتنا فأنه لا توجد بيانات معروفة حول التنوع الجيني لطفيلي مقوسة كوندي في الأغنام في العراق. لذا فأن الهدف من الدراسة هو للكشف عن السلالات (المورثات) التابعة للطفيلي المعزول من الأغنام في محافظة واسط، شرق العراق. تم جمع ٣١٥ عينة (دم ٣٠٠ و ١٥ نسيج مشيمة) من النعاج المجهضة، حيث فحصت أولا مصليا بواسطة فحص التلازن لاتكس، ومن ثم فحصت باستخدام تفاعل السلسة المتبلمرة في الوقت الحقيقي من خلال تضخيم الجين B1 لتأكيد الإصابة بطفيلي مقوسة كوندي. بعد ذلك، تم أختيار العينات الموجبة وفحصها لغرض التوصيف الوراثي حسب طريقة PCR-RFLP بالاعتماد على الجين SAG2. ظهر من بين ٣١٥ عينة تم فحصها، أن هنالك ١٠ عينات كانت موجبة لوجود الحمض النووي الخاص بمقوسة كوندي. وكشف فحص التنميط الجيني أن ٦٠٪ (٦/١٠) و ٣٠٪ (٣/١٠) و ١٠٪ (١/١٠) من العزلات المفحوصة تمثل الأنماط الوراثية الثاني والثالث والأول على التوالي. ويبدو أن النمط الثاني هو المهيمن في الأغنام في محافظة واسط، العراق.

Introduction

Toxoplasma gondii is a ubiquitous parasite. It can parasitized in all warm-blooded animals including man (1). Toxoplasmosis rarely causes clinical symptoms in healthy individuals, but, can cause severe effect in immunocompromised individuals (2). Wasit province is one of different provinces in Iraq having same food habits, where mutton is well-accepted for human consumption. Sheep are commonly infected with *T. gondii* (3), therefore considered a good source for human infection through ingestion of undercooked mutton containing *T. gondii*

tissue cysts (4). Three genetic lineages were described previously of *T. gondii*; they are type I, type II, and type III (5). Type I strain is more virulent in mice. Type II and type III strains are wide spread, whereas type II strain is more prevalent in human toxoplasmosis in congenital and acquired immune deficiency syndrome (AIDS) patients (6-8). Up to our knowledge no data are available about genetic diversity of *T. gondii* in sheep in Iraq. So, the aim of the present study is to detect the genotypes of *T. gondii* in sheep in Wasit province, Iraq.

Materials and methods

Three hundred and fifteen (blood 300 and 15 placenta's tissues) samples were obtained from aborted ewes from different regions in Wasit province throughout the period from October 2013 to May 2014.

Approximately 5ml of venous blood samples were drown from each aborted ewe and divided into two parts, 1 ml was transferred to tubes containing ethylene diamine tetra acetic acid (EDTA) for polymerase chain reaction (PCR) test and the rest 4 ml were centrifuged to get the sera kept after recovered in 1.5 ml tubes. Approximately 5 grams of placenta's tissue samples were collected from immediately aborted ewes. All samples were labeled individually and cooled with ice packs to maintain the temperature at 4°C during transport to the laboratory, when stored at -20°C until tested for *T. gondii*.

Genomic DNA extraction

DNA was extracted from frozen whole blood of positive latex agglutination test (LAT) cases and from all placentas' tissue samples using genomic DNA mini extraction kit (Geneaid, USA) with accordance to the manufacturer's instructions.

Detection of *T. gondii* by RT-PCR

RT-PCR was performed for rapid detection of *T. gondii* according to Lin *et al*. (9) using specific to amplification of *B1* gene which obtained from Bioneer company (South Korea) as forward primer (TCCCCTCTGCTGGCGAAAAGT), reverse primer (TCCCCTCTGCTGGAAAAGT) and *B1* probe (FAM-TCTGTGCAACTTTGGTGTATTCGCAG-TAMRA). The reaction was done using Accupower Dualstar TM q PCR premix (Bioneer, South Korea) according to the company instructions. The amplification steps included a first cycle of initial denaturation at 95°C for 5 minutes (min), 50 cycle of denaturation at 95°C for 15 seconds (sec), annealing / extension and detection (scan) at 60°C for 1 min.

Genetic characterization of *T. gondii*

The positive samples with RT-PCR test were subsequently genotyped by nested PCR-RFLP using genetic locus *SAG2* through separately amplified the 5' and 3' ends according to Howe *et al*. (10).

The amplification of 5' end was done by standard PCR for 40 cycles using the outer primers *SAG2*.F4 (5'-GCTACCTCGAAGGAACAC-3') and *SAG2*.R4 (5'-GCATCAACAGTCTTCGTTGC-3') at 65°C annealing temperature. One microliter (µl) of first PCR product was subsequently used as a template for nested PCR with inner primers *SAG2*.F (5'-GAAATGTTTCAGGTTGCTGC-3') and *SAG2*.R2 (5'-GCAAGAGCGAACTTGAACAC-3'). The 3' of *SAG2* locus was similarly amplified with outer primers *SAG2*.F3 (5'- TCTGTTCTCCGAAGTGACTCC-3') and *SAG2*.R3 (5'- TCAAAGCGTGCATTATCGC-3'). Also 1 µl of PCR amplicon was directly used for nested-PCR with inner primers *SAG2*.F2 (5'-ATTCTCATGCCTCCGCTTC-3') and *SAG2*.R (5'-AACGTTTCACGAAGGCACAC-3'). The protocol for temperature cycling was used as same as in both 5' and 3' end of *SAG2* loci, except for the annealing temperatures were applied as 65°C for 5' end and 63°C for 3' end of the gene. A *T. gondii* positive control (Genekam, Germany) was included in all nested PCR analysis. The nested- PCR products were purified by using PCR purification kit (Biobasic Inc. Canada), then 5 µl of the purified product of 5' and 3' ends were digested using *Sau3A*I and *Hha*I restriction enzymes (Biolab., UK) respectively in separated reactions according to the manufacturer's instructions. The digested products were electrophoresed on 2% agarose gel (10).

Results

One hundred blood samples out of 300 cases were positive by LAT and according to RT-PCR for detection of the *B1* gene of *T. gondii*; only 6 blood samples in addition to 4 placenta's tissue samples were successfully amplified.

All of these ten (6 blood and 4 placenta's tissue) samples were successfully amplified with nested PCR primer for *T. gondii SAG2* locus to produce product of 241bp and 221bp of 5' and 3' end respectively. The RFLP analysis of 5' and 3' nested PCR products revealed that type II strain was found in 6/10 (60%), type III in 3/10 (30%) and type I in 1/10 (10%) of isolates (Fig. 1 and 2; table 1). Type I strain results when no restriction occurs of both 5' and 3' regions of the *SAG2* gene by both *Sau3*AI and *Hha*I restriction enzymes, but type II strain result when *Hha*I enzyme cleavage 3' end product, while type III strain is revealed if the *Sau3*AI enzyme cleavage the 5' end product (10,11).

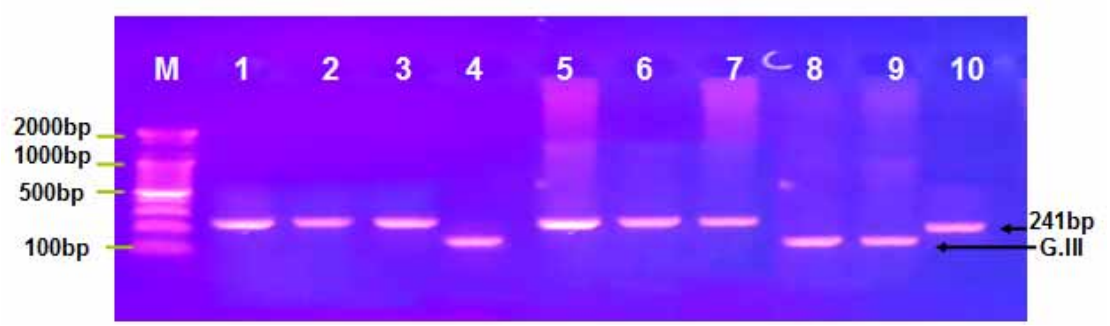

Figure 1: Positive RFLP-PCR result using Sau3AI restriction enzyme to produce genotype III. Where M: marker (2000-100bp), Lanes (4, 8 and 9): positive genotype III.

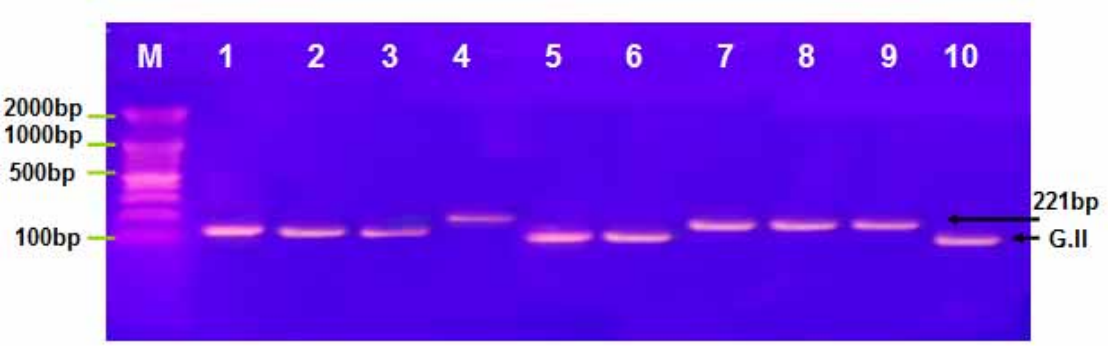

Figure 2: Positive RFLP-PCR result using *HhaI* restriction enzyme to produce genotype II. Where M: marker (2000-100bp), Lanes (1, 2, 3, 5, 6 and 10): positive genotype II.

Table 1: The strain types according to cleavage of restriction enzymes

No. of *T.gondii* isolates	5'end product cleavage by *Sau3*AI	3' end product cleavage by *Hha*I	Strain type
1	-	+	II
2	-	+	II
3	-	+	II
4	+	-	III
5	-	+	II
6	-	+	II
7	-	-	I
8	+	-	III
9	+	-	III
10	-	+	II

Discussion

This is the first attempt at genotyping *T. gondii* from sheep host in all Iraq.

Many previous studies conducted the nested PCR-RFLP method to determine the genotypes of the *T. gondii* at locus SAG2 (10-12).

In the present study 6/10 (60%) of *T. gondii* isolates from aborted ewes from Wasit in Iraq were type II, 3/10 (30%) were type III and 1/10 (10%) were type I strain.

The high frequency of the type II is in accordance with previous reports about sheep from France (13), Brazil (14), Italy (15) and Ethiopia (16). Type II strain is the most common strain in different host in Asia and Europe (17-19), the prevalence of type II might be due to its fitness and enhanced ability to outcompete other genotypes (20) as well as by its ability to form high numbers of cysts (5,21) in addition to being the most genotype identified in oocysts shed by definitive host (18). Type II strain has been shown to be associated with the majority of *T. gondii* infections in immunocompromised people and in congenital toxoplasmosis (6,22) and it is the most common isolated strain from human, sheep and pigs (23). In contrast there are some previous report referred to the fact that type I is the most common (24,25).

In the present study type III is found in 3/10 (30%) of isolates which is more than the result of Gebremedhin *et al*.

(16) who recorded only 9.09% of examined isolates had the type III strain. Hamilton *et al*. (26) reported that the type III was the predominance genotype of pigs, sheep and goats in West Indies. Many other studies recorded the type III strain but with low rates (27,28).

Regarding type I genotype which appeared in lowest rate 1/10 (10%) in our study, it is in accordance with many other studies which proved that type I strain of *T. gondii* is not predominant in sheep (26,27,29). The relative increase in percentage of type I strain in the current study may be attributed to the few numbers of tested positive samples.

Conclusion

The type II strain of *T. gondii* is the predominant type in the infected sheep in Wasit province, Iraq.

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