Toxoplasmosis in Cats: Serological and Molecular Study in Baghdad Province

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Abstract

The only hosts that can excrete resistant oocysts to environment are cats which play important role in the epidemiology of the Toxoplasma gondii. About one-third of the global population infected with T. gondii which is a major zoonotic agent. A total 141 blood samples collected from cats in Baghdad province, Iraq. Serological test on all serum samples were applied using latex agglutination test (LAT) were conducted to detect anti-T. gondii antibodies which showed 62.42% positive results. Age and sex regarded in this study were showed statistically no significant differences between both sexes or between different age groups. Although, the cats 6-10 years old showed highest positive results 74%. Nested polymerase chain reaction (nPCR) was also conducted on all serum samples targeting B1 gene showed 95.3% T. gondii infection.

Keywords: Toxoplasmosis, Cats, Serological study, Molecular study


Introduction

Toxoplasmosis is an infectious disease caused by obligatory intracellular protozoan Toxoplasma gondii. It is a wide spread in human and worm blood animals and considered as one of the most common parasite infection (Robert-Gangeux and Darde 2012). The disease is a worldwide in distribution and who has been exposed to this parasite about one-third of all human population. A wide range prevalence of toxoplasmosis is related to various factors including, sociocultural and nutritional habits, and geographical conditions (Barbosa et al., 2009). Three infective stages comprises life cycle of T. gondii which are the tachyzoites as invasive stage, bradyzoites as insisted stage and the sporozoites in environmental protected by oocyst wall. T. gondii can be transmitted vertically (across the placenta) and horizontally (tissue cysts, oocysts in water, soil or vegetation, organ transplants and blood transfusions) (Montoya and Liesenfeld, 2004, Al-Qassab et al., 2009). Cats act as a definitive host that shedding oocyte in feces and play a main role in spreading the disease (Dubey 2010). Soil, water and agriculture contaminated with feces act as a source of infection for domestic and wild animals. Humans infected through ingestion of oocytes in undercooked meat, contaminated vegetables, soil and water (Jones et al., 2014). Other infection pathways include transplacental transmission, blood
transfusion, organ transplantation, accidental inoculation in the laboratory and mechanical transmission by flies, cockroaches and earthworms (Bonfilio and Orefice, 2005; Hökelek, 2005). In Iraq, the seroprevalence of *T. gondii* used latex agglutination test was 30.4% in stray cats (Switzer, et al., 2013) and was 86% with indirect fluorescent antibody test (Hooshyar, et al., 2007). In Saudi Arabia, the seroprevalence of *T. gondii* for stray cats were (39%-90%) (Mohammed, et al., 2019; Al-Mohammed, 2011). In Turkey, seropositivity distribution range between 12.5% to 83.3%( Erkılıç, et al., 2016). The prevalence ranged from (95.5%) to (97.4%) in Egypt and 90% in Tehran, Iran with agglutination test (Al-Kappany et al 2011 &2010; Haddadzadeh, et al., 2006). Feral cats overall seroprevalence was 82% in Qatar (Boughattas et al., 2017). While in Ethiopia (Tiao, et al., 2013) recorded 85.4 % of feral cats as a seroprevalence.

The existences of *T. gondii* antibodies in serum samples of any host indicate the past infection of the host. Toxoplasmosis signs which are non specific clinically and characteristically not enough to give definite diagnosis. Humoral antibodies can be detected by many numerous serological methods such as latex agglutination test, enzyme linked immunosorbent assay and other may different serological procedures. When *T. gondii* antibodies fond in serum samples means the host may be exposed to past infection (Hill and Dubey 2002). For the diagnosis of *T gondii* infection in cats, the latex agglutination test is available commercially.

The presence of *T. gondii* in blood or tissue of mice confirmed by molecular analysis (PCR) when DNA extracted from infected mouse (Dubey et al., 2003).Copo-PCR was used by (Nabi, et al. 2018: Hanafiah, et al., 2018) and considered as a reliable way for *T. gondii* oocysts detection from feces in cats comparing to microscopic method (Nabi, et al., 2018). PCR as a nucleic acid based recognition way for DNA detection extracted from *T. gondii*. Target sequence B1 gene was widely used in PCR techniques which give best diagnostic resultsto identifying *T.gondii* infection in various body fluids (Alsaide, et al., 2019; Bakre, 2016; Burg, et al., 1989).Mawlood, et al., 2019 was recorded the *T. gondii* strain in stray cats in Iraq, Kurdistan as type III like strain. The aims of the study are to determine the seroprevalance of *T. gondii* in Iraqi cat’s also molecular detection and compare between LAT and PCR results.

**Materials and Methods**

All laboratory work was done in department of internal and preventive medicine, College of Veterinary Medicine, University of Baghdad. Blood samples were collected from stray cats in different places in Baghdad during the year 2016 and took one year.

**Blood collection:**

Blood samples were collected from 141 local Iraqicats (stray cats) by venipuncture from different places in Baghdad province including both sexes (55 male and 86 female) and different age groups. To control and easy manipulation, all cats were anesthetized using Ansatane® from Al-Hekma co., Jordan containing 0.01 thymol stabilized by Halothan 100% BP (Al-Kalidi, et al., 2017).
blood was stored at refrigerator until serum separation while two other ml was stored at -20 °C for DNA extraction.

Table (1) Distribution of samples according age and sex

<table>
<thead>
<tr>
<th>Age year</th>
<th>male</th>
<th>female</th>
<th>total</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-2</td>
<td>7</td>
<td>21</td>
<td>28</td>
</tr>
<tr>
<td>3-5</td>
<td>19</td>
<td>25</td>
<td>44</td>
</tr>
<tr>
<td>6-10</td>
<td>18</td>
<td>32</td>
<td>50</td>
</tr>
<tr>
<td>11-15</td>
<td>11</td>
<td>8</td>
<td>19</td>
</tr>
<tr>
<td>Total</td>
<td>55</td>
<td>86</td>
<td>141</td>
</tr>
</tbody>
</table>

Latex Agglutination Test:
Toxo- Latex commercially available from LiNEAR chemicals S.L Spain for determination of antibodies anti-Toxoplasma. The Toxo-latex is not species specific and is suitable for use in all species of animals. Procedure briefly 50 μl of Toxo-latex reagent was added to 50 μl of serum sample on slide, mixed and rotated on mechanical rotator (100 rpm) for 5 minutes. When degree of agglutination appeared mean positive reactive. Positive and negative control was included.

Molecular Diagnosis Method
Genomic DNA extraction
DNA was extracted from the whole blood samples collected in EDTA tubes using a commercial purification system (Wizard Genomic DNA purification kit, Promega, Madison, WI) following the manufacturer’s instruction for DNA purification from blood. The purity and concentration (ng/μl) of extracted DNA was measured using nanodrop spectrophotometer on 260-280 A. One microliter of elution buffer (AE) was used as a blank and one μl of DNA elution was loaded into a cuvette to measure the quantity of DNA in each extraction.

Primers and nested PCR
The primers used in this study depended on previously published work by Burg, et al., (1989). Two pairs of specific oligonucleotide primer targeting the gene (B1) of Toxoplasma gondii obtained from Integrated DNA Technologies Company (IDT) USA. Amplification and detection of T. gondii DNA were performed by nested PCR to amplify B1 gene fragment using different primers pairs as follows by two steps: first step forward 1 (F1) 5'-GGAACTGCATCCGTTCATGAG-3' and reverse 1 (R1) 5'-TCTTTAAAGCGTTCGTGGTC-3' amplify a 193 bp and second step F25'-TGATAGGTTGCAGTCACTG-3 and R2 ) 5'-GGCGACCAATCTGCGAATACACC-3' with 96 bp. Negative control reaction was set up containing all components of the reaction except distal template DNA which is replaced with distilled water. The amplification was carried out in TProfessional 96 thermal cycler (Biometra GmbH, Göttingen, Germany) at the following parameters: an initial cycle at 94 °C for 2 min, followed by 35 cycles of 94 °C for 30 second, 60 °C for 30 second, and 72 °C for 90 second. Final extension was performed at 72 °C for 2 min, while hold was at 4 °C. The second PCR step was done using 30 cycles under the same conditions and parameters as done in the first step.

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Results

Samples were collected from (141) cats from different areas in Baghdad including 55 (39%) male and 86 (61%) female and grouped according to age 1-2 years (28 cats), 3-5 years (44cats), 6-10 years (50cats) and 11-15 years (19 cats) as in table (2 and 3).

Table (2) Results of latex agglutination test of cats serum samples

<table>
<thead>
<tr>
<th>Sex</th>
<th>serum tested</th>
<th>No. of positive</th>
<th>Positive %</th>
<th>negative No.</th>
<th>negative %</th>
</tr>
</thead>
<tbody>
<tr>
<td>male</td>
<td>55</td>
<td>37</td>
<td>67.3</td>
<td>18</td>
<td>32.7</td>
</tr>
<tr>
<td>female</td>
<td>86</td>
<td>51</td>
<td>59.3</td>
<td>35</td>
<td>40.7</td>
</tr>
<tr>
<td>Total</td>
<td>141</td>
<td>88</td>
<td>62.4</td>
<td>53</td>
<td>37.6</td>
</tr>
</tbody>
</table>

Chi-Square (χ2) 0.908 NS with 1 d. f. and p.v.=0.341
NS= Non Significant

Table (3) results of latex agglutination test according to age group

<table>
<thead>
<tr>
<th>Age year</th>
<th>Serum samples</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>number</td>
</tr>
<tr>
<td>1-2</td>
<td>28</td>
</tr>
<tr>
<td>3-5</td>
<td>44</td>
</tr>
<tr>
<td>6-10</td>
<td>50</td>
</tr>
<tr>
<td>11-15</td>
<td>19</td>
</tr>
<tr>
<td>Total</td>
<td>141</td>
</tr>
</tbody>
</table>

Chi-Square (χ2) 5.607 NS with 3 d. f. and p.v.=0.132
NS= Non Significant

Positive results of toxoplasma latex agglutination test recorded in 88 serum samples representing 62.41% of total tested serum. The positive results of male serum samples was 37 out of 55 represented 67.27%, while the results of female serum samples was 51 out of 86 represented 59.3% as in table (2). Between genders no significant difference was recorded. According to age groups the results of positive latex-agglutination test revealed a difference in percent which ranged from 52.3 to 74 percentages. The highest percentage was in 6-10 years group recorded 74% out of 50 samples, while the lowest group was in 3-5 years with 52.3% (Table 3).

Toxoplasma DNA amplification was done by n-PCR and the bands of positive samples molecular size 96 bp showed when the second round of PCR carried out and were detected on agarose gel electrophoresis stained with ethidium bromide as shown in Fig. 1. Results of nPCR reveled very high positive percentages (68.08%) of T.gondii infection in Iraqi cats. High positive percentage was recorded in both sexes male and female 65.45% and 69.76% respectively (Table 4). Likewise PCR analysis according to age group uncover same elevated results 64.28% in 1-2 years, 65.90% in 3-5 years, and 63.15% in 11-15 years, with maximum percentage 72% in 6-10 years (Table 5).
Figure (1) 1.5% agarose gel electrophoresis for detection PCR product showed 96 bp fragment amplification 1-5, 7-14. M ismarker DNA ladder 1000 bp.

Table (4) Comparison between PCR analyses in gender in cats

<table>
<thead>
<tr>
<th>PCR Result</th>
<th>No. tested</th>
<th>positive %</th>
<th>negative %</th>
</tr>
</thead>
<tbody>
<tr>
<td>male</td>
<td>55</td>
<td>3665.45</td>
<td>1934.55</td>
</tr>
<tr>
<td>female</td>
<td>86</td>
<td>6069.76</td>
<td>2630.23</td>
</tr>
<tr>
<td>total</td>
<td>141</td>
<td>9666.09</td>
<td>4531.91</td>
</tr>
<tr>
<td>Chi-Square (χ²)</td>
<td>0.287 NS with 1 d. f. and p.v.=0.592 NS= Non Significant</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table (5) PCR analysis according to age group

<table>
<thead>
<tr>
<th>Age year</th>
<th>number</th>
<th>Positive</th>
<th>positive %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-2</td>
<td>28</td>
<td>19</td>
<td>64.28</td>
</tr>
<tr>
<td>3-5</td>
<td>44</td>
<td>29</td>
<td>65.90</td>
</tr>
<tr>
<td>6-10</td>
<td>50</td>
<td>36</td>
<td>72.00</td>
</tr>
<tr>
<td>11-15</td>
<td>19</td>
<td>12</td>
<td>63.15</td>
</tr>
<tr>
<td>Total</td>
<td>141</td>
<td>96</td>
<td>68.08</td>
</tr>
<tr>
<td>Chi-Square (χ²)</td>
<td>0.661 NS with 3 d.f. and p.v.=0.882 NS= Non Significant</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Discussion

Current study was showed the results of latex-agglutination test 62.4% these results agreed with the results of seroprevalence in Pakistan 60% (Maqbool, et al., 2002) and was higher than the results recorded (40%) in northern Iran (Sharif, et al., 2009) and results recorded (30.4%) in Iraq with LAT (Switzar, et al., 2013). Jalil and Alwan (2014) recorded seropositive ELISA 66% in stray cats in Baghdad while in Diwanyia-Iraq serological survey was showed 58.3% of IgG seropositive in domestic cats (Naser, etal., 2016) and 59.12% was in Iran (Khodaverdi and Razmi, 2019). In other studies
applied in countries which had border with Iraq were used different diagnostic methods recorded high positive results percentages as in Turkey (76.4%) T. gondii antibodies was in stray cats (Karatepe, et al., 2008) while in Iran, the seropositive prevalence ranged from 40% in northern Tehran to 86-90% in Tehran in stray cats (Sharif, et al., 2009; Hooshyar, et al., 2007; Haddadzadeh, et al., 2006). Due to variation in size of samples, cat’s age sample, methods of life of the cats and nutrition, and other circumstance make seroprevalence results difficult to compare (Tiao, et al., 2013).

The first researcher who used the B1 gene in PCR on detection of T. gondii DNA was Burge et al., 1989. Molecular research to detection Toxoplasma DNA in blood from cats seems to be very limited. Anyhow, many works were done as in blood collected from Saudi pregnant women and B1 gene was amplified in blood of (41%) by nPCR (Bin Dajem and Almushait, 2019) while (Hadi, 2016) detected T. gondii DNA in 65.15% of sheep serum samples. Molecular test nPCR revealed high results of T. gondii which was 68.08% in Baghdad province similar to results reported in Iran (Alkappany et al., 2011 and 2010). Many different works were established in Iraq, comparison results of different regions using different techniques in different animal species showed high percentage of infection. In sheep using LAT the overall prevalence was 83.3% in Duhok Governorate, 57.75% in Sulaimani, 100% in Erbil, Iraq (Mikail and Al-Barwary 2014; Kader, 2010). The percentage in goats was 60% with LAT and 83.69% with ELISA in Baghdad (Qazaz and Faraj, 2016).

The association between sex and seropositive cats to T. gondii was not significant in our study in agreement with results recorded elsewhere (Khodaverdi, and Razmi, 2019; Boughatlas et al., 2017; Cong, et al., 2016; Switzer, et al., 2013; Erkılıç et al., 2016; Karatepe, et al 2008; Hooshyer, et al., 2007) while high percentage of seropositive was detected in female cats (Boughatlas et al., 2017). Others reported significant higher seroprevalence in stray male cats (Cavalcante, et al., 2006).

No significant association between age and seropositive cats was observed in both tests even all of adults was seropositive (Erkılıç, et al., 2016; Switzer et al., 2013) which the last reference recorded 98.4% adult seropositive. The higher percentage was in 6-10 years old cats in accordance with (Mohammed, et al., 2019) and (Khodaverdi and Razmi 2019) recorded higher seroprevalence in adult cats over 3 years old. The diagnostic methods applied in other regions, different ways in management and breeds variation may play an important role in the difference of results among many references.

Finally, we can say the stray cats play an important role transmission the oocysts by seeding in feces causing environmental contamination. The study confirmed the occurrence of infection in stray cats in Baghdad province with high seropositivity percent while PCR was revealed a very high rate of cats infected with T. gondii. The association between sex and age with toxoplasma infection in stray cats was not significant statistically.
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