MOLECULAR DETECTION OF TOXOPLASMA GONDII IN LOCAL BREED DOMESTIC CHICKENS (GULLAS GULLAS DOMESTICUS) IN BAGHDAD CITY, IRAQ

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ABSTRACT

This study aimed to detect the infection rate of Toxoplasma gondii in 100 local breed domesticated chickens (Gullas gullas domesticus) by Real Time-PCR based on B1 gene and determined the effects of some factors (sex, age and areas) in this rate during the period 1/10/2019 till 31/3/2020. The total infection rate was 11% and a higher infection rate (17.24%) was found in females than males (2.38%) with significant (P≤0.01) difference. A higher infection rate (16.32%) was recorded in young (<6 months) females while no infection rate (0.00%) was found in young males (<6 months). The adult females (>6 months) were showed a higher infection rate (22.22%) than adult males 4.76% (>6 months) with significant (P≤0.01) difference. A higher infection rate (23.33%) was recorded in AL-Baia area followed by Al-Malhani (6.66%), while no infection rate (0.00%) was found in Baghdad Al-Jadida with significant (P≤0.01) difference.

Keyword: Toxoplasma, gondii, domestic chickens, Gallus, Real time PCR

Introduction

Toxoplasma gondii is one of the most world’s common protozoan parasite (Dubey and Beattie, 1988) cause a systemic disease (Siim et al., 1963; Kaneto et al., 1997; Tenter et al., 2000; Dubey, 2002). It is widespread zoonotic disease that infects all warm-blooded vertebrates (Smith and Reduck, 2000; Dubey, 2008; Dubey, 2010). The definitive hosts are domestic and wild cats (felids), which the sexual phase of the life cycle occurs in the epithelium of intestine ended by the shedding of unsporulated oocysts in the feces, while the intermediate hosts are animals or man (Dubey and Beattie, 1988; Hill and Dubey, 2002; Afonso et al., 2006; Dubey, 2010). The parasite has three infectious stages tachyzoites (rapidly multiplying and circulating), bradyzoites (tissue cysts) and sporozoites within oocysts (Hill and Dubey, 2002). In addition to naturally occurring toxoplasmosis (cases) in domestic birds, experimental studies have been carried out in many species such as white quails, Japanese quails, chickens, broilers, pigeons, turkeys, and pheasants (Boch et al., 1966; Bianeifiori et al., 1986; Dubey et al., 1993, 1993a; Kaneto et al., 1997). It has subclinical course in many avian species (Atasever et al., 2020). The clinical toxoplasmosis in chickens is consider as sciatic nerve neuritis, chorioretinitis and encephalitis (Hepding, 1939).

The definitive diagnosis of T. gondii is mainly established by parasitological, immunological and molecular tests (Villena et al., 2004) and the direct detection of parasite-specific DNA in biological samples using PCR-based molecular methods has gained popularity (Calderaro et al., 2006). These methods have proved to be simple, sensitive, reproducible, and have been applied to a variety of clinical samples from animals and humans (Bell and Ranford-Carteright, 2002; Contini et al., 2005; Bastien et al., 2007; Bessieres et al., 2009). Real-time PCR has been use to amplify and quantify DNA from the T. gondii B1 gene (Costa et al., 2000; Lin et al., 2000). It is a highly sensitive and specific method; but it is expensive, requires specialized detection systems; therefore may only be cost effective in laboratories where analysis of large numbers of samples are carried out (Nagy et al., 2007). Due to the no data available in the information about molecular diagnosis by real Time PCR of Toxoplasma gondii the infection in local breed domestic chickens this study was designed.

Materials and Methods

Animals, area and period of the study

One hundred local breed domestic chickens (Gullas gullas domesticus) were brought from the local markets (Abu-Ghurib, Al-Malhani, Baghdad Al-Jadida and Al-Baia) in Baghdad city during the period from 1/10/2019 till 31/3/2020.

Blood samples collection

About 2ml of jugular vein blood was collected of each bird by a sterile syringe into and transfer to EDTA tubes (Hendrix and Robinson, 2006) and kept in -20 °C till used for Real-Time PCR analysis.

DNA extraction

The DNA extraction from blood was done by using G-spin DNA extraction kit (Intron Biotechnology, cat. no.
17045) and the steps were follows the manufacture procedure and the primers of B1 gene and conditions that used for reactions were illustrated in table (1).

Table 1: The specific primers of B1 gene.

<table>
<thead>
<tr>
<th>Primers</th>
<th>Sequence</th>
<th>Tm (°C)</th>
<th>GC (%)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Forward</td>
<td>5'-TCCCCTCTGCTGGCGAAAAGT-'3</td>
<td>61.3</td>
<td>57.1</td>
<td>Al-Nasrawi et al. (2014)</td>
</tr>
<tr>
<td>Reverse</td>
<td>5'AGCGTTCGTGGTCAACTATCGATTG-3'</td>
<td>59.1</td>
<td>48</td>
<td>et al. (2014)</td>
</tr>
</tbody>
</table>

Agarose gel electrophoresis of DNA

The agarose gel has been made 1% (Sambrook et al., 1989). Three µl of the processor loading buffer (Intron / Korea) has been mixed with 5 µl of the supposed DNA to be electrophoresis. The process of loading added to the holes of the gel. An electric current of 7 v/2 had been exposed for 1-2 h till the tincture had reached to the other side of the gel. The gel had been tested by a source of the UV light with 336 nm after put the gel in pool contain 3µl red safe nucleic acid staining solution.

Components and concentrations of mixture for diagnose gene

The components and concentrations of mixture for diagnose gene illustrated in table (2)

Table 2: The components and concentration of mixture for diagnoses the B1 gene.

<table>
<thead>
<tr>
<th>Components</th>
<th>20 µL (Final volume)</th>
<th>Final concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sybrgreen Kappa Master Mix</td>
<td>10 µL</td>
<td>1X</td>
</tr>
<tr>
<td>Forward primer</td>
<td>1 µL</td>
<td>0.2µM</td>
</tr>
<tr>
<td>Reverse primer</td>
<td>1 µL</td>
<td>0.2µM</td>
</tr>
</tbody>
</table>

RT-PCR analysis for B1 gene

Amplification program for RT-PCR analysis (Lin et al., 2000) was illustrated in table (3).

Table 3: Amplification program of RT-PCR for B1 gene.

<table>
<thead>
<tr>
<th>No.</th>
<th>Amplification program</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>95.0°C-02:00x</td>
</tr>
<tr>
<td>2</td>
<td>95.0°C-00:30x</td>
</tr>
<tr>
<td>3</td>
<td>59.0°C-00:30x</td>
</tr>
<tr>
<td>4</td>
<td>72.0°C-01:00x</td>
</tr>
<tr>
<td>5</td>
<td>72°C-01:00x</td>
</tr>
</tbody>
</table>

Statistical analysis

Chi-square test ($\chi^2$) was used to compare the significance difference between the parameters (SAS, 2012).

Results

The total infection rate

The total infection rate of *Toxoplasma gondii* by Real Time-PCR in the domesticated local breed chickens (*Gallus gallus domesticus*) was 11% (11/100) in Baghdad city (Table 4, Fig. 1).

Table 4: Total infection rate of *Toxoplasma gondii* in local breed chickens.

<table>
<thead>
<tr>
<th>Test</th>
<th>No. of samples examined</th>
<th>Positive</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Real time PCR</td>
<td>100</td>
<td>11</td>
<td>11</td>
</tr>
</tbody>
</table>

Fig. 1: The Positive results of *Toxoplasma gondii* in chickens by Real Time PCR in Baghdad city.

The effect of sex in infection rate of *Toxoplasma gondii* in chickens

A higher infection rate 17.24% (10/58) of *Toxoplasma gondii* was recorded in females, while the lower infection rate 2.38% (1/42) was found in males with significant (P≤0.01) difference (Table 5)

Infection rate of *Toxoplasma gondii* in chickens according to sex and age

The infection rate of *Toxoplasma gondii* in domesticated local breed chickens (*Gallus gallus domesticus*) according to sex and age was showed a higher infection rate 16.32% (8/49) in young female chickens and a lower infection rate 0.00% (0/21) was found in young male chickens. Also, adult female chickens were showed a higher...
infection rate 22.22% (2/9), than adult male chickens which showed a lower infection rate 4.76% (1/21) with significant (P≤0.01) difference. (Tab. 6)

The effects of areas in the infection rate of *Toxoplasma gondii* in chickens.

There was an effect of areas on the infection rates of *Toxoplasma gondii* of domesticated local breed chickens

Table 5 : The effect of sex in infection rate of *Toxoplasma gondii* in chickens.

<table>
<thead>
<tr>
<th>Sex</th>
<th>No. of samples examined</th>
<th>Positive</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males</td>
<td>42</td>
<td>1</td>
<td>2.38</td>
</tr>
<tr>
<td>Females</td>
<td>58</td>
<td>10</td>
<td>17.24</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>11</td>
<td>11</td>
</tr>
</tbody>
</table>

**P≤0.01**

Table 6 : Infection rate of *Toxoplasma gondii* in chickens according to sex and age by Real Time -PCR.

<table>
<thead>
<tr>
<th>Age/sex</th>
<th>No. of samples examined</th>
<th>Positive</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 6 months</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>21</td>
<td>0</td>
<td>0.00</td>
</tr>
<tr>
<td>Females</td>
<td>49</td>
<td>8</td>
<td>16.32</td>
</tr>
<tr>
<td>&gt; 6 months</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>21</td>
<td>1</td>
<td>4.76</td>
</tr>
<tr>
<td>Females</td>
<td>9</td>
<td>2</td>
<td>22.22</td>
</tr>
</tbody>
</table>

**P≤0.01**

Table 7 : The infection rate of *Toxoplasma gondii* in chickens according to the areas.

<table>
<thead>
<tr>
<th>Areas</th>
<th>No. of samples examined</th>
<th>Positive</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Al-Baia</td>
<td>30</td>
<td>7</td>
<td>23.33</td>
</tr>
<tr>
<td>Baghdad Al-Jadida</td>
<td>15</td>
<td>0</td>
<td>0.00</td>
</tr>
<tr>
<td>Abu-Ghurib</td>
<td>25</td>
<td>2</td>
<td>8.00</td>
</tr>
<tr>
<td>Al-Malhaini</td>
<td>30</td>
<td>2</td>
<td>6.66</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>11</td>
<td>11</td>
</tr>
</tbody>
</table>

**P≤0.01**

Discussion

Toxoplasmosis is one of the most common human infections throughout the world infection is more common in warm climates and at lower altitudes than in cold climates and mountainous regions (James, 2003). Gondim et al. (2010) was mentioned the detection of *T. gondii* DNA in seronegative birds that was confirmed the importance of other diagnostic techniques to complement serological examination of birds infected with this parasite. Generally, the routine diagnosis of infection is commonly performed by serological tests Modified Agglutination Test (MAT) and Indirect Fluorescent Antibody Test (IFAT) for detection of specific antibodies (Dubey et al., 2002, 2003a, b, 2005a, 2007). The molecular methods have are more sensitive, specific and take less time compared to other assays (Villena et al., 2004). Real-time PCR has been used to amplify and quantify DNA from the B1 gene (Dubey and Beattie, 1988; Costa et al., 2000; Lin et al., 2000). The result of the presents study were showed that all over infection rates in chickens by RT-PCR 11% that was agree or disagree with some previous studies in the world such as India 17.9% (Sreekumar et al., 2003), Brazil 38% (Dubey et al., 2007a), Nicaragua 85% (Dubey et al., 2006), China 11.4% in free ranging chickens and 4.1% of caged chickens, Egypt 18.7% was found antibodies in chickens from the slaughterhouses by using MAT (Deyab and Hassanain, 2005), and Thailand 64% in free ranging chickens by using fluorescent antibody test (IFAT) and 47.2% that was reported by El-Massary et al. (2000) and Zhu et al. (2008). Zhao et al. (2012) was referred that the seropositive rates of infection very high from 30-50% in free-range chickens or maybe reached 100% in backyard chickens (Dubey, 2010; Dubey et al., 2010), that difference in the infection rate of parasite was mentioned before in naturally infected poultry varies greatly (Siim et al., 1963; Devada et al., 1998; El-Massary et al., 2000; Dubey, 2010) and Shokri et al. (2017) was found in the field the overall prevalence included chicken 20%, pigeons 8% and sparrows 15%. The high prevalence of *T. gondii* in all the areas showed that the free-range chickens are a major reservoir for *T. gondii* parasites. The free-range chicken in the study area had free access to habitats around homesteads where they scavenged for feed which mainly included left overs, grass, and insects. (Mose et al., 2016) or in most developing countries, the free-range chickens are slaughtered at home or in unsupervised slaughterhouses and their viscera such as heads are left for scavengers that can include cats and other chickens (Dubey et al., 2012). The variation in the infection rates may due to the distribution of domestic cats, which are the major source of contamination to the environment due to the greatest formation of oocysts in these animals, which excrete millions of them after ingesting only one bradyzoite or tissue cyst (Dubey and Frenkel, 1972;
Dubey, 2001). On the same way, the wall of oocyst is adapted very well to protect it from the damage to extreme makes the parasite resilient for the environment and it can be survived in the moist environment for more than one year (Mai et al., 2009). The soil contamination with oocysts is an important factor in the development of disease in free-range poultry (Dubey, 2010). The prevalence of parasite in free-ranging chickens is a good indicator of the prevalence of parasite oocysts in the soil because they feed from the ground (Ruiz and Frenkel, 1980; Dubey et al., 2005). Free ranging chickens play an important role in the epidemiology of parasite in the rural environment because they are clinically resistant to it and live longer; cats that fed naturally infected chickens tissues can shed millions of oocysts (Dubey, 2002a); the oocysts in soil may do not stay there, but the invertebrates such as flies, cockroaches, dung beetles and earthworms can be mechanically spread and/or even carry them onto food, and the infection rate in cats reflects the infection in local avian and rodent population, because they are thought be infected by eating these animals and release more oocysts in the environment, more prey animals will become infected, and the result is increase the cats infection (Dubey and Beattey, 1988). The sporogony of oocysts occurs outside the host and leads to the development of infectious oocysts that remain viable in the environment for months to years (Lelu et al., 2012). Sporulated oocysts are very resistant to environmental conditions, and remain infective in humid soil for more than 18 months; but they don’t survive long under cold or dry conditions (Dubey et al., 2000). Although, the oocysts disseminated throughout the premises (Levine, 1973) and ingesting of them in water, soil or feed is probably the most common route for infection in non-carnivorous mammals and birds; meat-producing animals can show a very high seroprevalence throughout the world may be up to 100% (Tenter et al., 2000), for that domestic breeding birds and poultries are less infected than free ranging or industrial breeding since they are not allowed to contact with infective oocysts or feline (Holsbak, 2012). It is also known that felids are generally excellent predators, thus contributing to maintenance and success of T. gondii in the environment and birds can be considered as an important reservoirs of T. gondii as they are often hunted by felids (Dubey, 2006). Also the tissues of infected chickens are a source for cats infection (Ibrahim et al. 2009; Shokri et al., 2017). In conclusion, T. gondii was distributed with a high infection rate in local breed domestic chickens in Baghdad city.

References


