Detection of Toxoplasma gondii IgG antibodies in Nigerian Free-range Chickens using Indirect Fluorescent Antibody Test (IFAT)

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Key Words: Free-range chickens; Toxoplasma gondii; IFAT; Nigeria; Serology

**ABSTRACT:** The detection of Toxoplasma gondii antibodies in free-range chickens (FRC) has become a method for monitoring soil contamination with T. gondii oocysts shed in cat faeces. Although several methods have been employed for T. gondii antibody detection in chicken sera, limited information is available on the use of indirect fluorescent antibody test (IFAT) for this purpose. This study was conducted to determine the occurrence of IgG antibodies to T. gondii in the sera of 241 FRC randomly obtained from 10 local government areas (LGAs) of Oyo state, southwestern Nigeria using IFAT. Antibodies specific for T. gondii were detected in 26 (10.8%) sera with titre of 1:25 in 26 samples, and 1:50 in 5 samples. Seropositive FRC were detected in all 10 LGAs of Oyo State. There was no significant association between T. gondii seroprevalence and age, gender or source of the FRC. The results of the present study show that the IFAT is valuable in detecting antibodies to T. gondii in FRC sera and that infection of these chickens with T. gondii is widespread in the study area. Adequate precautions should be taken during preparation of FRC from the study area for food, as they could serve as potential source of infection for humans.

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1. INTRODUCTION

Toxoplasma gondii infection is ubiquitous and is one of the most widespread zoonoses affecting all warm-blooded animals with significant impact on public health and animal production (Dubey and Beattie, 1988; Dubey, 2010b). T. gondii infection in chickens has been reported worldwide (Dubey, 2010a), mostly without clinical symptoms and in some cases may cause mortality (Dubey et al., 2007; Dubey, 2010a). The detection of T. gondii in free-range chickens (FRC) has become a method for monitoring soil contamination with T. gondii oocysts because they feed on the ground, thereby having access to the hidden faeces of cats (Dubey, 2010b). The consumption of T. gondii-infected chicken meat has been reported as a source of infection for humans and other animals including cats (Dubey, 2010a). Hence, there is a need for efficient and standardized ready-to-use assay for detecting the parasite in domestic chickens. Such test will not only help to better understand the epidemiology and transmission of T. gondii to various host populations, it will also aid the design of prevention and control measures, especially in developing countries where the practice of rearing chickens on free-range is widespread.

Majority of reports on detection of T. gondii in chickens have been based on serology using either commercial or in-house tests (Dubey, 2010a). Polymerase chain reaction and bioassay of tissue in mice have also been carried out (Aigner et al., 2010; Hamidinejat, 2014). The serological tests used to detect antibodies to T. gondii in sera of chickens include enzyme-linked immunosorbent assay (ELISA), modified agglutination test (MAT), latex agglutination test (LAT), complement fixation test (CFT), indirect fluorescent antibody test (IFAT), complement inhibition test (CIT), indirect haemagglutination antibody test (IHAT) and the Sabin-Feldman dye test (DT) (Dubey, 2010a). While the ELISA and MAT have been demonstrated to be efficient in chicken and other animals (Dubey, 2010a; Bahnasset al, 2015; Ayinmode et al., 2015), the DT, CFT and IHAT have been found to be of little value for detecting T. gondii antibodies in the sera of chickens and limited information is available on the accuracy of IFAT and LAT (Dubey, 2010a).

The prevalence of antibodies to T. gondii in domestic chickens varies considerably among laboratories especially with the availability of several commercial kits. Many laboratories also develop their own in-house assays with varying cut-off values and interpretation. All these practices
contribute to the inability to compare the results of serological testing for *T. gondii* antibodies. Previous work done on detection of *T. gondii* antibodies in Nigerian chickens utilized the IHAT and MAT (Aganga et al., 1984; Ayinmode and Dubey, 2012; Ayinmode and Olaosebikan, 2014). This study was conducted to determine the occurrence of antibodies (IgG) to *T. gondii* in FRC in Oyo state, southwestern Nigeria using IFAT.

2. MATERIALS AND METHODS

2.1 Sampling location

Serum samples were randomly collected from FRC bought at markets in about 50 towns and villages located across 9 local government areas of Oyo state, Southwestern Nigeria. Oyo state lies between latitude 7° N to 9° N and longitude 2°8° E to 4.5° E, and covers an area of approximately 28,454 square kilometers within the rainforest area of West Africa (Blexxonmak, 2010).

2.2 Sample collection

Blood samples were collected from the wing vein of the chickens and separated sera were stored at -20°C till serology was conducted. Data on age, sex, and sources of chickens were obtained.

2.3 Laboratory analysis

*Indirect Fluorescent Antibody Test (IFAT)*

The IFAT for detection of *T. gondii* IgG antibodies in the chicken sera was performed by carrying out a two-fold serial dilution of each serum in phosphate buffered saline (PBS) to obtain 1:25, 1:50, 1:100, 1:200 and 1:400 dilutions. Slides coated with formalinized tachyzoites obtained from tissue culture were dipped in acetone and kept frozen for 10 minutes. The slides were then transferred into PBS for 10 minutes and excess fluid was removed using an aspirator. 10 µl of diluted serum was added into each well on the labelled slides. The slides were incubated for 30 minutes at 37°C. Excess serum was aspirated and the slides were dipped in FA buffer for 10 minutes. After aspiration of excess FA buffer from the slides, 10 µl rabbit anti-chicken IgG conjugate (Rockland Inc, USA) diluted 1:200 and 2% Evans blue dye were added to each well and this was also incubated for 30 minutes at 37°C. The excess fluid on the slides was aspirated and the slides were dipped in FA buffer and PBS for 10 minutes, respectively. Excess fluid was again aspirated and the slides were allowed to air-dry. Mounting fluid was added to the wells on the slides, cover – slip was applied and the slides were viewed under a fluorescent microscope (X.1000). Samples were considered positive when fluorescein-stained, banana-shaped tachyzoites were observed. The cut-off titre was set at 1:25.

2.4 Statistical Analysis

Statistical analysis of the association of seroprevalence of *T. gondii* antibodies in chicken with risk factors (sex, age and source of chickens) was performed using the Fisher’s Exact test with Graphpad Quickcals (Graphpad softwares Inc., La Jolla, USA). A p-value < 0.05 was considered statistically significant.

3. RESULTS

A total of 26 out of 241 (10.8%) serum samples tested positive for *Toxoplasma gondii* IgG antibodies using the IFAT with a titre of 1:25 obtained in 26 samples and 1:50 in 5 samples. No sample was positive at 1:100, 1:200 and 1:400 dilutions. Antibodies to *T. gondii* were detected in FRCs from all local government areas (Table 1). Seropositivity in female and male chickens was 11.4% (16/140) and 9.9% (10/101), respectively while it was 10.4% (24/183) and 8.6% (7/85) among chickens less than 24 months and greater than 24 months, respectively. Samples from urban and rural areas had prevalence of 9.1% (15/164) and 14.3% (11/77) for *T. gondii* antibodies, respectively. There was no statistically significant association between prevalence of *T. gondii* antibodies and sex, age and source of FRC (Table 2).

4. DISCUSSION

The detection of *T. gondii* antibodies in this study using IFAT show that FRC in Oyo state, Southwestern Nigeria are exposed to infection sources for the parasite.

Comparison of our findings with other surveys where IFAT was used show that the seroprevalence of 10.8% is lower than 53.6% obtained in Brazil (Brandao et al., 2006), 40.5% in Costa Rica (Abrahams-Sandi et al., 2005) and 21.7% in India (Sreekumaret al., 2001). Several factors like cat population, and disparity in sensitivity, specificity, protocols and cut-offs of tests have been known to affect the prevalence of *T. gondii* infection worldwide. For instance, the cut-off of the present study was 1:25 while those for the cited reports from Brazil, Costa Rica and India were 1:64, 1:16 and 1:8, respectively. This and other factors make it difficult to compare the outcome of IFAT for *T. gondii* antibodies in chickens between different laboratories. Our study, to the best of our understanding, is the first to utilize IFAT for detection of *T. gondii* in Nigerian FRC; other studies utilized MAT (Ayinmode and Dubey, 2012) and IHAT (Aganga and Belino, 1984) on chicken sera.
Table 1: Seroprevalence of *Toxoplasma gondii* in different Local Government Areas of Oyo State, Nigeria

<table>
<thead>
<tr>
<th>LGA</th>
<th>Number Tested</th>
<th>Number Positive</th>
<th>Prevalence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Akinyele</td>
<td>28</td>
<td>3</td>
<td>10.7</td>
</tr>
<tr>
<td>Atiba</td>
<td>25</td>
<td>2</td>
<td>8.6</td>
</tr>
<tr>
<td>Atisbo</td>
<td>28</td>
<td>3</td>
<td>10.7</td>
</tr>
<tr>
<td>Egbeda/Ona-Ara</td>
<td>36</td>
<td>3</td>
<td>8.3</td>
</tr>
<tr>
<td>Ibarapa North</td>
<td>16</td>
<td>1</td>
<td>6.3</td>
</tr>
<tr>
<td>Idi</td>
<td>27</td>
<td>2</td>
<td>7.4</td>
</tr>
<tr>
<td>Ogbomosho North</td>
<td>20</td>
<td>2</td>
<td>10</td>
</tr>
<tr>
<td>Oyo West</td>
<td>33</td>
<td>6</td>
<td>18.2</td>
</tr>
<tr>
<td>Saki East</td>
<td>38</td>
<td>4</td>
<td>10.5</td>
</tr>
<tr>
<td>Total</td>
<td>241</td>
<td>26</td>
<td>10.8</td>
</tr>
</tbody>
</table>

Table 2: Seroprevalence of *T. gondii* in Chickens of different sexes, ages and sources (cut-off titre of 1:25)

<table>
<thead>
<tr>
<th>Factor</th>
<th>Category</th>
<th>No Tested</th>
<th>No positive</th>
<th>Prevalence (%)</th>
<th>Odds Ratio</th>
<th>95% CI</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>&lt;24 months</td>
<td>183</td>
<td>9</td>
<td>10.4</td>
<td>0.548</td>
<td>0.176-1.707</td>
<td></td>
</tr>
<tr>
<td></td>
<td>&gt;24 months</td>
<td>58</td>
<td>5</td>
<td>8.6</td>
<td>1.824</td>
<td>0.586-2.678</td>
<td>0.334</td>
</tr>
<tr>
<td>Sex</td>
<td>Male</td>
<td>101</td>
<td>10</td>
<td>9.9</td>
<td>0.852</td>
<td>0.370-1.963</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>140</td>
<td>16</td>
<td>11.4</td>
<td>1.174</td>
<td>0.509-2.707</td>
<td>0.834</td>
</tr>
<tr>
<td>Source</td>
<td>Rural</td>
<td>77</td>
<td>11</td>
<td>14.3</td>
<td>1.656</td>
<td>0.722-3.799</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Urban</td>
<td>164</td>
<td>15</td>
<td>9.1</td>
<td>0.604</td>
<td>0.263-1.386</td>
<td>0.267</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>241</td>
<td>26</td>
<td>10.8</td>
<td></td>
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</tr>
</tbody>
</table>

Our recent study on the same set of FRC samples using MAT(titer ≥ 1:20) yielded a higher seroprevalence of 40.4% (Ayinmode and Olaosebikan 2014). This suggests that the MAT is more sensitive than IFAT. Furthermore, the MAT is less cumbersome, faster, cost-effective, requires less skills and has the advantage of being able to screen more samples at a time. However, the use of specific conjugate for the IFAT suggests that it is more specific and possibly more reliable than the MAT. Further studies are therefore needed to evaluate the effectiveness of the IFAT for detection of *T. gondii* antibodies in chicken sera.

The present study revealed no statistically significant association between occurrence of *T. gondii* antibodies and factors like gender, age and source of birds. This finding contrast with the reports of some previous studies, which showed that prevalence of *T. gondii* antibodies was higher in adult chickens due to repeated exposure during their longer lifetime (Dzitkoet., al, 2006; More et al., 2012). Many factors such as variability in levels of environmental contamination with oocysts from cats may account for this disparity. Our study also showed that birds from all the 50 surveyed towns and villages in Oyo state had been exposed to *T. gondii* infection. This suggests widespread contamination of the environment with *T. gondii* oocysts and showed that FRC obtained from these locations could be source of *T. gondii* infection for humans and cats, the final host. Since viable *T. gondii* has been isolated from FRC with low antibody titre (Dubey, 2010a), consumption of chickens with tissue cyst can result in infection.

5. Conclusion

The present survey, which reports the use of IFAT for surveillance of *T. gondii* antibodies in sera of Nigerian FRC, showed that *T. gondii* infection is widespread in FRC in all the surveyed local government areas of Oyo state. The outcome of this study is of public health concern and indicates the
need for prevention and control of *T. gondii* infection in the study area where FRC are increasingly preferred as source of meat for human consumption.

6. Acknowledgement
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7. REFERENCES


