Comparative Analysis of Toxoplasmosis in Farm Animals by Indirect Hemagglutination Assay and Enzyme linked Immunosorbent Assay

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Key words:
Najran, Saudi Arabia, Seroprevalence, Indirect Hemagglutination test, enzyme-linked immunosorbent assay, Toxoplasmosis.

ABSTRACT:
A sero-prevalence survey of Toxoplasmosis in domestic animals (sheep, goats and camels) was conducted in the Najran region of Saudi Arabia from August to November 2014 to determine the status of the disease, as well as to evaluate the diagnostic performance of the serological tests used. A total of 263 sera were tested, 85 sheep; 88 goats and 90 camels, for the presence of Toxoplasma gondii antibodies using Indirect Hemagglutination test (IHAT) and enzyme-linked immunosorbent assay (ELISA). IHAT showed the higher prevalence of Toxoplasmosis (43.5% in sheep flowed by 31.8% in goats and 24.4% in camels) as compared with ELISA. On the other hand, ELISA showed the higher prevalence of Toxoplasmosis in sheep (45.9%) as compared with IHAT(43.5%). When the data from the IHAT was compared with that of the ELISA test, which was used as a reference test for toxoplasmosis, IHAT had the highest sensitivity (88.1%). It could be concluded that the IHAT and ELISA tests are efficient diagnostic tools for detection and selective diagnosis of Toxoplasmosis. Also high seroprevalence of Toxoplasma gondii in studied animals indicated the importance of these animals as the main source of human infection.

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1. INTRODUCTION

Toxoplasma gondii is a worldwide parasite which infects any nucleated cells of birds and mammals including human. In human, it may cause severe medical complications in immune-compromised individuals or in congenitally acquired cases. The parasite can cause congenital abnormalities such as abortion, chorioretinitis, hydrocephaly and jaundice (Montoya and Remington, 2008).

T. gondii infection in livestock such as sheep, goats and camels is responsible for considerable economic losses due to abortion of fetus. Infections with this zoonotic parasite are acquired mostly by consumption of undercooked or raw meat, which contains tissue cysts, or water contaminated with oocysts of T. gondii. Additionally, infection not only results in significantly reducing reproduction and hence causing economic losses, but also has implications for public health, since consumption of contaminated meat or milk can facilitate zoonotic transmission.

T. gondii oocysts are shed by domestic cats and other felines resulting in widespread contamination of the environment. Domestic cats are most likely the main source of contamination as they are common and produce large numbers of oocysts (Dubey, 2004).

Prevention and control of the prevalence of T. gondii and effective prevention of toxoplasmosis in animals and humans require good diagnostic or testing methods that are appropriate for the specific species. There are many testing methods for T. gondii detection in animals and humans (Hill et al., 2006; Moghazy et al., 2011), however, serological tests have some distinct advantages that have been described elsewhere (Dubey et al., 2005; Gamble et al., 2005; Shaapan et al., 2008).

The current study was conducted to determine the seroprevalence Toxoplasma gondii in farm animals in Najran State. Moreover, the efficiency of
ELISA and IHAT for detecting *T. gondii* antibodies in the sera of naturally infected animals was compared, and their intrinsic agreement was also evaluated through analysis of \(k\) statistics. Furthermore, the sensitivity and specificity of IHAT and ELISA methods were also evaluated.

2. MATERIALS AND METHODS

2.1. Study area

The present investigation was conducted in the Southern region of Saudi Arabia, particularly in the Najran area which lies between 17° 30' 20” North and 44° 11’ 3” East. Almost 60% of the population lives in rural areas in close contact with livestock.

2.2. Animal samples

A total of 263 animal serum samples were collected from Najran slaughterhouses including 85 sheep, 88 goat and 90 camels between August to November 2014. The university ethical board gave permission to conduct the study within the institutional research mandate as stipulated by the National Ethical Board. All serum samples were stored at -20° C until use.

2.3. Serological examination

The collected sera were examined for detection of *T. gondii* antibodies using the indirect haemagglutination assay (IHAT) with a commercially available kit (TOXO- HAI, FUMOUZE laboratories, France) according to the manufacturer’s instructions. In brief, sera were added to 96 well V bottomed polystyrene plates, and diluted in a four-fold series from 1:80 to 1:2560. The plates were shaken for 2 min and then incubated at 37° C for 2 h without shaking. The test was considered positive when a layer of agglutinated erythrocytes was formed in wells at dilutions of 1:80 or higher, and positive and negative controls were included in each test. All positive samples that reacted with titres > 1:80 were tested once again after treatment with 2-mercaptoethanol (2-ME) to detect IgM antibodies (Camargo et al., 1978).

On the other hand, enzyme-linked immunosorbent assay (ELISA) was also used for the evaluation of anti-Toxoplasma IgG and IgM antibodies with a Vircell anti-*T. gondii* ELISA set (G1027 and M1027, Granada, Spain). Antibody levels were evaluated by following the manufacturer's instructions on the set at the laboratory of the department of Applied Medical Sciences, Community College, Najran University, Saudi Arabia.

2.4. Statistical analysis

The significance of differences was analyzed using chi-square \((\chi^2)\) using the Statistical Package for Social Science version 15.0 (SPSS Inc., Chicago, IL), and \(p<0.05\) was considered significant. Additionally, the degree of agreement between the results from the 2 tests was quantified using \(k\) statistics and the accuracy of the IHAT test in detecting exposure to *Toxoplasma* was evaluated in comparison to the ELISA, and measured using the relative sensitivity and specificity.

3. RESULTS

3.1. Serological examination for the detection of *T. gondii* antibodies

In the present study, most of studied animals (86%) had antibody titer of 1:80. The highest rate of *Toxoplasma* infection (45.9%) was found in sheep followed by goats (29.5%) and camels (21.1%) (Table 1). This marked difference was found to be statistically significant \((\chi^2=37.74, p<0.0001)\), while there was no significant difference detected between positive and negative results when comparing IHA and ELISA results with chi-square test \((p=0.78)\) and the strength of agreement between the 2 tests was considered to be substantial\((k =0.79)\).

Of the 85 samples randomly selected from sheep, 37 (43.5%) were positive by IHAT and 39 (45.9%) were positive by ELISA (Table 1). The \(\chi^2\) test suggested that the positive rate of antibodies to *T. gondii* in naturally infected sheep was not differed significantly between the two methods \((\chi^2=0.095, P>0.05)\). However, \(k\) analysis demonstrated that the two methods had a high degree of agreement in detecting *T. gondii* infection in sheep (\(k=0.87\)) (Table 4).

Among the 88 serum samples from goats, 28 (31.8%) were positive by IHAT and 26 (29.5%) by ELISA (Table 1). The \(\chi^2\) test suggested that there was no significant difference between the positive rates of *T. gondii* antibodies detected by the two methods (\(\chi^2=0.107\)). The corresponding \(k\) value decreased to 0.67, although it still demonstrated a substantial agreement between the two methods in detecting *T. gondii* infection in goats (Table 4).

From the 90 sera samples collected from camels, 22 (24.4%) were found positive by IHAT and 19 (21.1%) by ELISA (Table 1). The \(\chi^2\) test suggested that there was no significant difference between the positive rates of *T. gondii* antibodies detected by the two methods \((\chi^2=0.284)\). The corresponding \(k\) value decreased to 0.65, while it still demonstrated a substantial agreement between the two methods in detecting *T. gondii* infection in camels (Table 4).

Table 2 and 3 summarized the results of Prevalence of *T. gondii* antibodies (IgG & IgM) among farm animals sera using IHAT and ELISA assays.
### Table (1): Indirect hemagglutination assay (IHA) and enzyme-linked immunosorbent assay (ELISA) seroprevalence of *Toxoplasma gondii* in farm animals from the Najran region, Saudi Arabia

<table>
<thead>
<tr>
<th>Animal species</th>
<th>IHA</th>
<th>ELISA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number of examined</td>
<td>Number of positive</td>
</tr>
<tr>
<td>Sheep</td>
<td>85</td>
<td>37</td>
</tr>
<tr>
<td>Goats</td>
<td>88</td>
<td>28</td>
</tr>
<tr>
<td>Camels</td>
<td>90</td>
<td>22</td>
</tr>
<tr>
<td>Total</td>
<td>263</td>
<td>87</td>
</tr>
</tbody>
</table>

<sup>a,b,c</sup> Value with different superscript in the same column differ at *p*<0.05

### Table (2): Prevalence of *T. gondii* antibodies (IgG & IgM) among farm animals sera using IHA test

<table>
<thead>
<tr>
<th>Animal species</th>
<th>IHAT</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number of examined</td>
<td>Number of positive</td>
<td>Percentage of positive (%)</td>
</tr>
<tr>
<td></td>
<td>Sheep</td>
<td>85</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>Goats</td>
<td>88</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>Camels</td>
<td>90</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>263</td>
<td>63</td>
</tr>
</tbody>
</table>

### Table (3): Prevalence of *T. gondii* antibodies (IgG & IgM) among farm animals sera using ELISA assay

<table>
<thead>
<tr>
<th>Animal species</th>
<th>ELISA</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number of examined</td>
<td>Number of positive</td>
<td>Percentage of positive (%)</td>
</tr>
<tr>
<td></td>
<td>Sheep</td>
<td>85</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td>Goats</td>
<td>88</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>Camels</td>
<td>90</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>263</td>
<td>59</td>
</tr>
</tbody>
</table>

### Table (4): Diagnostic performance of indirect hemagglutination assay (IHA) and enzyme-linked immunosorbent assay of farm animals with antibodies against *Toxoplasma gondii*.

<table>
<thead>
<tr>
<th>Evaluation of IHA test</th>
<th>IgG</th>
<th>IgM</th>
<th>IgG</th>
<th>IgM</th>
<th>IgG</th>
<th>IgM</th>
<th>IgG</th>
<th>IgM</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>sheep</td>
<td>goat</td>
<td>Camel</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sensitivity %</td>
<td>93.1</td>
<td>85.7</td>
<td>88.2</td>
<td>77.7</td>
<td>76.9</td>
<td>83.3</td>
<td>88.1</td>
<td>81.8</td>
<td></td>
</tr>
<tr>
<td>Specificity %</td>
<td>94.6</td>
<td>97.4</td>
<td>95.8</td>
<td>96.2</td>
<td>93.5</td>
<td>97.6</td>
<td>94.6</td>
<td>97</td>
<td></td>
</tr>
<tr>
<td>Accuracy %</td>
<td>94.1</td>
<td>96.5</td>
<td>94.3</td>
<td>94.3</td>
<td>91.1</td>
<td>96.7</td>
<td>93.1</td>
<td>95.8</td>
<td></td>
</tr>
<tr>
<td><em>κ</em> value</td>
<td>0.87&lt;sup&gt;***&lt;/sup&gt;</td>
<td>0.75&lt;sup&gt;†&lt;/sup&gt;</td>
<td>0.67&lt;sup&gt;†&lt;/sup&gt;</td>
<td>0.50&lt;sup&gt;**&lt;/sup&gt;</td>
<td>0.65&lt;sup&gt;†&lt;/sup&gt;</td>
<td>0.80&lt;sup&gt;†&lt;/sup&gt;</td>
<td>0.79&lt;sup&gt;†&lt;/sup&gt;</td>
<td>0.74&lt;sup&gt;†&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

<sup>*</sup> Substantial agreement  <sup>**</sup> Moderate agreement  <sup>***</sup> Almost perfect agreement
Comparative studies of the different serological tests used for detection of T. gondii antibodies

Sensitivity and specificity calculations of the serological tests for IgG by using ELISA assay as a reference test revealed that IHAT had the highest sensitivity (93.1%) was observed in sheep, while the highest specificity (95.8%) was in goat. On the other hand; the highest sensitivity (85.7%) of the IHAT IgM was in sheep, while the highest specificity (97.6% and 97.4%) of the IHAT IgM was in camel and sheep respectively. IHAT IgM test was highly sensitive and specific in acute sheep toxoplasmosis (Table 4).

4. DISCUSSION

Animal species for this study were chosen according to their role in human life in Najran State, Saudi Arabia. Sheep were the main source of meat, and their viscera were common protein particularly among those who eat raw meat. Cameline unboiled milk or raw liver are consume by nomads around Najran State. Furthermore, people in Najran State are recognized as consumer of goats' meat.

Generally, the higher infection rate observed in this study may be attributed to the feeding habits of farm animals and the stray cats that entered their environment, making them more likely to contract T. gondii oocysts. This is in agreement with results obtained by Lunden et al. (1994).

The prevalence of T. gondii in slaughter sheep were studied in many countries, the percent were range between 3% in Pakistan using latex agglutination test (LAT) (Zaki, 1995) to highest percent in Indonesia (60%) by using IHAT (Iskandar, 1998). In Norway using ELISA recorded 18% (Skjerve et al., 1996). In Saudi Arabia, Egypt, and Djibouti where using IHAT, the prevalence 39% in Saudi Arabia (Amin and Morsy, 1997), in Egypt 29% (EL Ridi et al., 1990), and 10% in Djibouti (Chantal et al., 1994).

In goats, the seroprevalence were studied in many countries like Pakistan (52%) by using LAT (Tasawar et al., 2011), Djibouti (6.4%) by using IHAT (Chantal et al., 1994), Iran (15%) by using Enzyme-Linked Immuno-sorbent Assay, ELISA (Ghazaei, 2006) and Ethiopia, 1989, (11.6%) by using IHAT (Chantal et al., 1994).

On the other hand, the prevalence of camel toxoplasmosis compared to the results of previous studies as reported in Sudan (20%) by using LAT (Khalil and Elrayah, 2011), Egypt (30.7%) by using Modified Agglutination Test, MAT (Shaapan and Khalil, 2008), and Turkey (90.9%) by using Sabin-Feldman Dye Test (Utuk et al., 2012).

The differences in prevalence of toxoplasmosis in different animal species may be explained by differences in susceptibility to infection (Fayer, 1981). While, generally the difference of prevalence rates around the world might be due to serological methods used or difference of breed or difference of sex.

In regard to prevalence of T. gondii antibodies (IgG & IgM) among farm animals sera using IHAT and ELISA assays, the presence of anti-IgM seroprevalence (Acute phase) reflects the risk among animals with a recent infection who might transfer the parasite to men and/or another farm animals, as in the acute stage of the disease they are shedding T. gondii tachyzoites in all body fluids, including milk. Similar figures were previously reported by Dubey and Lin (1994). Furthermore, A negative IgM with a positive IgG result indicates infection at least 1 year previously. A positive IgM result may indicate more recent infection or may be a false positive reaction (Tekkesin, 2012)

The variation between the results obtained from the studied animals sera using different serological tests might be due to differences in the sensitivity and specificity of the serological tests used. ELISA demonstrates great sensitivity, is quantitative, low cost and may be automatically adopted, but requires further refinement with regard to procedures and standardization of the antigen used (Dubey et al., 1995). Regarding IHAT, it has the highest sensitivity in comparison to ELISA tests, is easy to perform, and does not require sophisticated equipment.

5. CONCLUSION

It could be concluded that domestic animals (sheep, goats and camels,) in Najran are widely infected by Toxoplasmosis; sheep is more prevalent. The study recommends the need for further researches in the whole country using different serological tests and to determine the impact of these findings on the human population. The continued rise of public awareness of zoonotic potential of Toxoplasmosis is also recommended. Additionally, the results of the present work demonstrate the benefits of using the more sensitive and somewhat specific IHAT for the detection of T. gondii antibodies in studied animal sera.

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6. REFERENCES


