Seroprevalence of toxoplasmosis in humans and animals in Pakistan: a systematic review and metadata analysis

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Abstract

Objective: To review the seroprevalence of toxoplasmosis in Pakistan.

Method: The systematic review comprised search on Science Direct, Google Scholar, PubMed and Scopus databases for studies related to the seroprevalence of toxoplasmosis in Pakistan published between 2006 and 2020 which used serological diagnostic tests to detect Toxoplasma gondii. Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines were used throughout the review and statistical analysis was done using forest plot and random effect model.

Results: Of the 7093 human studies initially found, 20 (0.28%) were reviewed. Of the 16,432 animal studies, 16 (0.09%) were selected for detailed review. The pooled seroprevalence of toxoplasmosis in humans, calculated in this review was found as (76%) (95% confidence interval: 69-83%). Seroprevalence of human toxoplasmosis was higher in Khyber Pakhtunkhwa (31.7%) than Punjab (20.4%). Pooled seroprevalence in animals calculated in this review was found as (69%) (95% confidence interval: 64-74%). Seroprevalence in animals was higher in Khyber Pakhtunkhwa (44.7%) than Punjab (29.4%).

Conclusions: The seroprevalence of toxoplasmosis in both humans and animals should be studied it other parts of Pakistan as well.

Key Words: Livestock, Humans, Random-effect model, Toxoplasma gondii, Seroprevalence.

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Introduction

Toxoplasmosis is a zoonotic disease caused by parasite Toxoplasma (T.) gondii. The modes of transmission of the parasite include the consumption of the meat of sheep, cattle, goat, and pigs having bradyzoites of T. gondii, and the consumption of milk of goat, sheep and cattle, or contaminated chicken eggs.1 More commonly, humans acquire infection through unintentional ingestion of unwashed fruits, vegetables, water contaminated with oocysts excreted by infected felines.2 The principal reservoir for T. gondii is wild and domesticated cats, and the oocysts are produced in cats through sexual stages of their lifecycle. During pregnancy, T. gondii can cause abortion in the pregnant host, an infection of the placenta and in the foetus, leading to miscarriage, foetal mummification and or death in all types of mammalian hosts and among animals, especially in sheep and cattle.3 T. gondii has a huge zoonotic potential and causes infection in humans across the globe. High seroprevalence in the population leads to higher ratio of infections.4 It can cause different neurological disorders in immunocompromised or human immunodeficiency virus/acquired immunodeficiency syndrome (HIV/AIDS) patients.5

Pooled seroprevalence of toxoplasmosis in Pakistan has been determined by compiling data. It has been found that 30-50% of human population is infected with T. gondii, worldwide.6 Almost 20 million new cases of congenital and acquired toxoplasmosis were reported in 2010.7 On the basis of public and animal health, interaction of microorganisms and environment, horticulture, gardening, trade effects as well as socio-economic impacts, T. gondii is graded as one of the top 10 food-borne parasites.8 T. gondii is identified through direct methods, like microscopy and detection of deoxyribonucleic acid (DNA) by polymerase chain reaction (PCR), while the segregation of T. gondii is done by various bioassays.9 T. gondii is also identified through indirect methods, like the detection of antibodies against T. gondii in the serum of humans and animals.10 Various serological techniques, such as Sabin-Feldman Dye test, enzyme-linked immunosorbent assay (ELISA), latex agglutination test (LAT), indirect haemagglutination test (IHAT) and rapid diagnostic test strip (RDTs), have been employed for the identification of T. gondii antibodies in humans and animals.11 In Pakistan, various epidemics cause morbidity and death in the livestock sector. Due to these diseases, Pakistan faces an estimated loss of around PKR79 billion.12 Toxoplasmosis is one of the cosmopolitan parasitic
of 7145 individuals were part of these studies, and 1813 (25.37%) were seropositive for toxoplasmosis. A broad difference was seen in the estimation of seroprevalence among various studies (Q=863.34; df=19; p<0.001; I2=97.8%; 95% CI: 97.5-98.1%). The pooled seroprevalence of toxoplasmosis was determined as 76% (95% CI: 69-83%) (Figure 2). Seroprevalence was higher in KP (31.7%) compared to Punjab (20.42%) (Figure 3). The seroprevalence was detected using LAT in (10%) studies (Table 1).

Of the 16,432 animal studies, 16(0.09%) were selected for detailed review (Figure 4). Of them, 4(25%) were done in KP and 12(75%) in Punjab. A total of 8,519 animals were part of these studies, and 2,647(31%) of them were seropositive. A wide range of difference was seen in the seroprevalence estimation among the studies (Q=504.5; df=19; p<0.001; I2=95.4%; 95% CI: 94.5-96.1%). The pooled seroprevalence of toxoplasmosis in animals was determined as 69% (95% CI: 64-74%) (Figure 5).
Table 1: Summary of the reviewed.

<table>
<thead>
<tr>
<th>Serial No.</th>
<th>Areas/Regions</th>
<th>Provinces</th>
<th>Total individuals</th>
<th>Positive Cases</th>
<th>Prevalence (%)</th>
<th>Diagnostic Methods</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Chiniot, Faisalabad, Jhang and Okara</td>
<td>Punjab</td>
<td>593</td>
<td>44</td>
<td>7.4%</td>
<td>ELISA</td>
<td>30</td>
</tr>
<tr>
<td>2</td>
<td>Mardan</td>
<td>Khyber Pakhtunkhwa</td>
<td>180</td>
<td>73</td>
<td>40.6%</td>
<td>ELISA</td>
<td>31</td>
</tr>
<tr>
<td>3</td>
<td>Pothwar region (Islamabad, Rawalpindi, Attock, Chakwal, and Jhelum)</td>
<td>Punjab</td>
<td>1659</td>
<td>338</td>
<td>20.4%</td>
<td>ELISA</td>
<td>32</td>
</tr>
<tr>
<td>4</td>
<td>Abbottabad, and Mansehra</td>
<td>Khyber Pakhtunkhwa</td>
<td>500</td>
<td>124</td>
<td>24.8%</td>
<td>RDTS, ELISA</td>
<td>33</td>
</tr>
<tr>
<td>5</td>
<td>Bannu</td>
<td>Khyber Pakhtunkhwa</td>
<td>150</td>
<td>2</td>
<td>1.3%</td>
<td>ELISA</td>
<td>34</td>
</tr>
<tr>
<td>6</td>
<td>Bannu</td>
<td>Khyber Pakhtunkhwa</td>
<td>170</td>
<td>4</td>
<td>2.3%</td>
<td>ELISA</td>
<td>35</td>
</tr>
<tr>
<td>7</td>
<td>Charsadda</td>
<td>Khyber Pakhtunkhwa</td>
<td>200</td>
<td>69</td>
<td>34.5%</td>
<td>LAT</td>
<td>36</td>
</tr>
<tr>
<td>8</td>
<td>Matta and Upper Swat</td>
<td>Khyber Pakhtunkhwa</td>
<td>360</td>
<td>170</td>
<td>47.2%</td>
<td>LAT</td>
<td>37</td>
</tr>
<tr>
<td>9</td>
<td>Swabi</td>
<td>Khyber Pakhtunkhwa</td>
<td>100</td>
<td>12</td>
<td>12%</td>
<td>RDTS</td>
<td>38</td>
</tr>
<tr>
<td>10</td>
<td>Multan</td>
<td>Punjab</td>
<td>403</td>
<td>71</td>
<td>17.6%</td>
<td>ELISA</td>
<td>39</td>
</tr>
<tr>
<td>11</td>
<td>Chitralt</td>
<td>Khyber Pakhtunkhwa</td>
<td>300</td>
<td>74</td>
<td>24.7%</td>
<td>RDTS</td>
<td>40</td>
</tr>
<tr>
<td>12</td>
<td>Mohmand Agency</td>
<td>Khyber Pakhtunkhwa</td>
<td>580</td>
<td>165</td>
<td>28.4%</td>
<td>LAT</td>
<td>41</td>
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<tr>
<td>13</td>
<td>Malakand Agency</td>
<td>Khyber Pakhtunkhwa</td>
<td>420</td>
<td>276</td>
<td>65.7%</td>
<td>RDTS, LAT, and ELISA</td>
<td>42</td>
</tr>
<tr>
<td>14</td>
<td>Muzaffargarh</td>
<td>Punjab</td>
<td>150</td>
<td>63</td>
<td>42%</td>
<td>LAT</td>
<td>43</td>
</tr>
<tr>
<td>15</td>
<td>Muzaffargarh</td>
<td>Punjab</td>
<td>100</td>
<td>37</td>
<td>37%</td>
<td>LAT</td>
<td>44</td>
</tr>
<tr>
<td>16</td>
<td>Bhawal Nagar, Shuja Abad, and Multan</td>
<td>Punjab</td>
<td>550</td>
<td>162</td>
<td>29.5%</td>
<td>LAT</td>
<td>45</td>
</tr>
<tr>
<td>17</td>
<td>Lahore</td>
<td>Punjab</td>
<td>300</td>
<td>34</td>
<td>11.3%</td>
<td>LAT</td>
<td>46</td>
</tr>
<tr>
<td>18</td>
<td>Dera Ghazi Khan</td>
<td>Punjab</td>
<td>200</td>
<td>59</td>
<td>29.5%</td>
<td>LAT</td>
<td>47</td>
</tr>
<tr>
<td>19</td>
<td>Kohat</td>
<td>Khyber Pakhtunkhwa</td>
<td>180</td>
<td>26</td>
<td>14.4%</td>
<td>ELISA</td>
<td>48</td>
</tr>
<tr>
<td>20</td>
<td>Lahore</td>
<td>Punjab</td>
<td>50</td>
<td>10</td>
<td>20%</td>
<td>LAT</td>
<td>49</td>
</tr>
</tbody>
</table>

Figure 3: Topographical distribution of human toxoplasmosis in different regions. 66.
seroprevalence of toxoplasmosis in animals was higher in KP (44.7 (%)) compared to Punjab (29.4 (%)) (Figure 6).

LAT, ELISA and IHAT were used for the diagnosis of toxoplasmosis in animals (Table 2).

Discussion

Besides causing a high disease load in humans, *T. gondii* also causes considerable economic loss to the livestock sector. The pooled seroprevalence of toxoplasmosis in humans and animals determined in the current systematic review was found as 76% and 69%, respectively. The findings are like those in Central America, Latin America, Europe and Asia (75-85%), while northern Europe and the United States have shown low seroprevalence.

In Pakistan, the average seroprevalence of toxoplasmosis in humans was 20% between 2006 and 2020. No data were reported from the Sindh, Balochistan, Gilgit-Baltistan and Azad Jammu and Kashmir (AJK) in this regard. Thereafter, the seroprevalence of toxoplasmosis in humans has ranged between 1.3-65.7%. Also, in Pakistan, the average seroprevalence of toxoplasmosis in animals was 31% between 2006 and 2020. In other studies, it has ranged from 10% to 55.4%.

Seroprevalence of toxoplasmosis in the region has been reported in humans and animals to be 39.3% and 31%, respectively, in Iran, and 12.5% and 23.7%, respectively, in China.

Toxoplasmosis is associated with numerous risk factors. Abundance of cats is one of the vital sources of the spread of *T. gondii* infection in various regions of Pakistan.
the sporulated oocysts can live for months and even for years in the soil. Moreover, earthworms, flies and beetles can escalate the oocysts and pass these on to the food chain.26

In Pakistan, such high rate of seropositivity of T. gondii may be due to lack of awareness, lack of education among people, and improper hygienic conditions of the population. Lack of awareness about the infection, its sources of spread, its routes of transmission and ignorance of the outcomes of the diseases are important factors in the risk of infections.27 The chances of infections can be reduced by increasing the level of education and spreading awareness among the people.28

Various serological methods are regularly used for the identification of T. gondii antibodies.28 The current review noted that the most common methods used were LAT and ELISA. However, Toxoplasma immunoglobulin M (IgM) chemiluminescence ELISA (CLIA) is a robotic test that is performed in a very short time but is not a regular method used in either private or public laboratories in Pakistan.

Epidemiological investigations reckon important information for devising precautions for the control of toxoplasmosis. Awareness of seroprevalence of

<table>
<thead>
<tr>
<th>Sr No.</th>
<th>Area/ City</th>
<th>Year of Study</th>
<th>Target Animal</th>
<th>Total Animals</th>
<th>Infected with Toxoplasmosis</th>
<th>Seroprevalence (%)</th>
<th>Diagnostic Method</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Thal and Cholistan</td>
<td>2019</td>
<td>Camel</td>
<td>897</td>
<td>360</td>
<td>40.1%</td>
<td>Indirect ELISA</td>
<td>50</td>
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<tr>
<td>2</td>
<td>Multan</td>
<td>2018</td>
<td>Sheep and Goats</td>
<td>250</td>
<td>107</td>
<td>42.8%</td>
<td>LAT</td>
<td>51</td>
</tr>
<tr>
<td>3A</td>
<td>Charsadda</td>
<td>2018</td>
<td>Cows</td>
<td>139</td>
<td>77</td>
<td>55.4%</td>
<td>LAT</td>
<td>52</td>
</tr>
<tr>
<td>3B</td>
<td>Charsadda</td>
<td>2018</td>
<td>Goats</td>
<td>149</td>
<td>62</td>
<td>41.6%</td>
<td>LAT</td>
<td>52</td>
</tr>
<tr>
<td>4A</td>
<td>Multan and Khanewal Districts</td>
<td>2016</td>
<td>Sheep</td>
<td>500</td>
<td>168</td>
<td>33.6%</td>
<td>LAT</td>
<td>52</td>
</tr>
<tr>
<td>4B</td>
<td>Multan and Khanewal Districts</td>
<td>2016</td>
<td>Sheep</td>
<td>500</td>
<td>137</td>
<td>27.4%</td>
<td>ELISA</td>
<td>53</td>
</tr>
<tr>
<td>5A</td>
<td>Bhalwal, Kotmomin, Sahiwal, Shahpur, Silanwali and Sargodha Districts</td>
<td>2016</td>
<td>Sheep</td>
<td>470</td>
<td>123</td>
<td>26.2%</td>
<td>ELISA</td>
<td>54</td>
</tr>
<tr>
<td>5B</td>
<td>Bhalwal, Kotmomin, Sahiwal, Goahpur, Silanwali and Sargodha Districts</td>
<td>2016</td>
<td>Goats</td>
<td>530</td>
<td>227</td>
<td>42.8%</td>
<td>ELISA</td>
<td>54</td>
</tr>
<tr>
<td>6A</td>
<td>Cholistan Desert and Agricultural Areas of Rahim Yar Khan and Rajan Pur (Punjab) Pakistan</td>
<td>2016</td>
<td>Sheep</td>
<td>335</td>
<td>125</td>
<td>37.3%</td>
<td>LAT</td>
<td>55</td>
</tr>
<tr>
<td>6B</td>
<td>Cholistan Desert and Agricultural Areas of Rahim Yar Khan and Rajan Pur (Punjab) Pakistan</td>
<td>2016</td>
<td>Goats</td>
<td>865</td>
<td>252</td>
<td>29.1%</td>
<td>LAT</td>
<td>55</td>
</tr>
<tr>
<td>7</td>
<td>Faisalabad, Lahore and Gujranwala</td>
<td>2015</td>
<td>Horses (Equines)</td>
<td>272</td>
<td>91</td>
<td>33.5%</td>
<td>LAT</td>
<td>56</td>
</tr>
<tr>
<td>8A</td>
<td>Pothwar region (Islamabad, Rawalpindi, Attock, Chakwal and Jhelum)</td>
<td>2015</td>
<td>Sheep</td>
<td>413</td>
<td>75</td>
<td>18.2%</td>
<td>ELISA</td>
<td>57</td>
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<tr>
<td>8B</td>
<td>Pothwar region (Islamabad, Rawalpindi, Attock, Chakwal and Jhelum)</td>
<td>2015</td>
<td>Goats</td>
<td>419</td>
<td>60</td>
<td>14.3%</td>
<td>ELISA</td>
<td>57</td>
</tr>
<tr>
<td>9</td>
<td>Bahawalpur</td>
<td>2014</td>
<td>Camels</td>
<td>100</td>
<td>10</td>
<td>10%</td>
<td>LAT</td>
<td>58</td>
</tr>
<tr>
<td>10</td>
<td>Faisalabad</td>
<td>2014</td>
<td>Backyard Poultry</td>
<td>300</td>
<td>109</td>
<td>36.3%</td>
<td>LAT</td>
<td>59</td>
</tr>
<tr>
<td>11A</td>
<td>Pothwar region (Islamabad, Rawalpindi, Attock, Chakwal and Jhelum)</td>
<td>2014</td>
<td>Cattle</td>
<td>400</td>
<td>79</td>
<td>19.7%</td>
<td>ELISA</td>
<td>60</td>
</tr>
<tr>
<td>11B</td>
<td>Pothwar region (Islamabad, Rawalpindi, Attock, Chakwal and Jhelum)</td>
<td>2014</td>
<td>Buffaloes</td>
<td>422</td>
<td>64</td>
<td>15.2%</td>
<td>ELISA</td>
<td>60</td>
</tr>
<tr>
<td>12A</td>
<td>District Mardan</td>
<td>2013</td>
<td>Goats</td>
<td>350</td>
<td>148</td>
<td>42.3%</td>
<td>IHA</td>
<td>61</td>
</tr>
<tr>
<td>12B</td>
<td>District Mardan</td>
<td>2013</td>
<td>Sheep</td>
<td>290</td>
<td>128</td>
<td>44.1%</td>
<td>IHA</td>
<td>61</td>
</tr>
<tr>
<td>13</td>
<td>Multan</td>
<td>2011</td>
<td>Goats</td>
<td>200</td>
<td>104</td>
<td>55%</td>
<td>LAT</td>
<td>62</td>
</tr>
<tr>
<td>14</td>
<td>Dera Ghazi Khan, Multan and Khanewal</td>
<td>2010</td>
<td>Sheep</td>
<td>518</td>
<td>103</td>
<td>19.9%</td>
<td>LAT, ELISA</td>
<td>63</td>
</tr>
<tr>
<td>15A</td>
<td>Rahim Yar Khan</td>
<td>2009</td>
<td>Goats</td>
<td>110</td>
<td>28</td>
<td>25.4%</td>
<td>LAT</td>
<td>64</td>
</tr>
<tr>
<td>15B</td>
<td>Rahim Yar Khan</td>
<td>2009</td>
<td>Sheep</td>
<td>90</td>
<td>10</td>
<td>11.2%</td>
<td>LAT</td>
<td>64</td>
</tr>
<tr>
<td>16</td>
<td>Cholistan</td>
<td>2015</td>
<td>Sheep</td>
<td>335</td>
<td>125</td>
<td>37.3%</td>
<td>LAT</td>
<td>65</td>
</tr>
</tbody>
</table>
Toxoplasma, disease problems and precautionary methods can lead to potent incidence curtailment. 29

The current systematic review has some limitations, like missing information about the seroprevalence of toxoplasmosis in different regions of Pakistan because of the absence of relevant studies, use of different methods for the identification of toxoplasmosis with variable levels of sensitivities and specificities, and diverse epidemiological outcomes. All these factors can have an influence on the overall seroprevalence rate mentioned in the review.

Conclusion

There is a need to create awareness among the masses to control toxoplasmosis in Pakistan. The relevant authorities should execute a screening programme in all provinces using standardised methods in order to estimate the actual prevalence and risk factors of toxoplasmosis in the country. Finally, there is a dire need to perform studies in Balochistan, Sindh, GB, and AJK regarding the seroprevalence of toxoplasmosis in human and animal populations.

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