Seroprevalence and Associated Risk Factors of Toxoplasmosis in Sheep and Goats in Pothwar Region, Northern Punjab, Pakistan

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Abstract.- Present study was designed to find out the prevalence and associated risk factors of Toxoplasma gondii infection in sheep and goats present in northern parts of Punjab province, Pakistan. For this purpose serum samples collected from 413 sheep and 419 goats were tested for detection of anti-Toxoplasma-IgG and IgM antibodies by using enzyme linked immunosorbent assay. The results showed that overall prevalence of T. gondii antibodies in sheep and goats was 18.16% (75/413) and 14.32% (60/419) respectively. Prevalence of IgG antibodies was 15.49% (64/413) in sheep and 11.93% (50/419) in goats while IgM antibody prevalence was 3.39% (14/413) and 2.86% (12/419) in sheep and goats respectively. Infection was more common in female and older animals. Other statistically significant risk factors in sheep were poor hygienic conditions (OR = 4.91, p<0.01), presence of cats (OR = 2.08, p<0.05), extensive farming practice (OR = 2.19, p<0.05), flock size larger than 50 individuals (OR = 4.24, p<0.01) and pregnancy (OR = 2.50, p<0.05). Similarly statistically significant risk factors in goats were poor hygienic condition (OR = 2.06, p<0.05), usage of outdoor water source (OR = 2.06, p<0.01), presence of cats (OR = 2.03, p<0.05), extensive farming practice (OR = 2.25, p<0.05) and flock size larger than 30 (OR = 4.24, p<0.05) and 50 (OR = 6.82, p<0.01) individuals. Seroprevalence was significantly high in salt range sheep as compared to other breeds of sheep (OR = 5.51, p<0.01). Results indicate that T. gondii infection is widely prevalent in sheep and goats of northern Punjab, Pakistan and may have important consideration for livestock industry and public health.

Key words: Seroprevalence, Toxoplasama gondii, sheep, goats, serology, Pothwar region.

INTRODUCTION

Toxoplasmosis is a widely distributed parasitic infection in small ruminants caused by a protozoan Toxoplasma gondii. It is not only responsible for economic losses due to abortion and neonatal deaths in infected animals, but also have zoonotic significance as it may be transmitted to humans via contaminated meat and milk (Dubey, 1996; Pal et al., 1995,1996; Ghoneim et al., 2009). Sheep and goats may acquire infection by grazing on contaminated pastures and/or through drinking oocyst contaminated water of cat origin (Vesco et al., 2007). In small ruminants, toxoplasmosis is a leading source of abortion, still births and neonatal deaths, however, sub-clinical infection may also occur in adult animals (Buxton, 1990; Hassig et al., 2003). Congenital transmission occurs when female animals get infection during pregnancy and acute infection may cross placenta and multiply in fetal tissue resulting in abnormal development and abortion. Prevalence rates vary greatly in both species in different parts of the world mainly due to difference in farming practices and environmental conditions (Dubey and Beattie, 1988; Tenter et al., 2000).

Epidemiological studies of toxoplasmosis have been carried out in many countries owing to its importance in livestock sector (Figliuolo et al., 2004). In Pakistan, breeding of sheep and goats is an important source of milk and meat production. Apart from commercial husbandry practices, these animals are also reared by poor farmers because of their low cost maintenance and short term return. Studies conducted in southern parts of the country have confirmed the prevalence of T. gondii in sheep and goats (Ramzan et al., 2009; Lashari and Tasawar, 2010; Tasawar et al., 2011). No such report of T. gondii infection in small ruminants exists for northern Punjab (Pothwar Plateau) which has unique geography and climate as compared to...
other parts of the country. Therefore, keeping in view the importance of the infection, a seroepidemiological study was conducted to work out the seroprevalence of *T. gondii* infection and associated risk factors in sheep and goats raised in northern Punjab, Pakistan.

**MATERIALS AND METHODS**

*Study area and animal management*

This study was carried out in Pothwar region, Pakistan. Pothwar plateau is located in northern parts of the Punjab province between 32°-30’ to 34° northern latitudes and 71°-45’ to 73°-45’ eastern latitudes. It occupies a total area of 23,160 sq kilometers and has a total human population of 7.5 millions. It includes Islamabad capital territory along with four districts namely Rawalpindi, Attock, Chakwal and Jhelum. Agriculture is mainly dependent on rainfall which averages 370-500 mm annually. Most of the rainfall occurs in northwestern parts while southwestern parts are usually arid.

Sheep and goats grazing in Pothwar region of Pakistan follow extensive and semi-extensive farming practices. Sheep and goat are generally kept separate but they closely follow each other sharing the grazing lands. The flocks of sheep and goats are taken out in the morning for grazing along the communal lands, road sides and land along foot-hills and brought back to their kraal in the evening.

*Study animals, sampling and serum preparation*

Sheep and goats were randomly selected from different farms located in Pothwar region. The area was divided into 20 clusters and 20-25 samples were taken from each cluster for each species. A total of 831 small ruminants (413 sheep and 419 goats) were screened for the presence of *Toxoplasma* specific IgG and IgM antibodies between September, 2011 and December, 2012. Three breeds of sheep (Salt Range, Awassi and Afghani) and three breeds of goats (Beetal, Jattal and Teddy) along with their crosses were included in the study. About 3-4 ml blood samples were collected from jugular vein in 5 ml vacutainers without anticoagulant. Blood samples were immediately transported to PMAS-Arid Agriculture University, Rawalpindi. Serum was removed by centrifugation at 3000 rpm for 15 minutes. All the serum samples were stored at –20°C until used for *Toxoplasma*-specific IgG and IgM antibodies detection. Age was determined from animal record kept at farms. Additional data regarding management practice, source of water and presence of cats etc was obtained through surveys and interviews.

*Enzyme-linked immunosorbent assay (ELISA)*

*Toxoplasma*-specific IgG-antibodies were measured by a commercial ELISA Kit (ID Screen Toxoplasmosis Indirect ® (ID-VET Company, France) according to manufacturer’s instruction. Results were also interpreted according to the instructions of the manufacturer.

*Statistical analysis*

Odds ratios were calculated by using SPSS statistical package by comparing infected and non-infected animals for working out different determinants of *T. gondii* infection such as breeds, sex, age, management practice, source of water, presence of cats, reproductive status and flock size.

**RESULTS**

In current study, out of total 413 sheep sampled, 75 were found positive giving an overall prevalence of 18.16%. *Toxoplasma*-specific IgG antibodies were found in 64 (15.49%) while IgM antibodies were found in 14 (3.39%) sheep. Three sheep were positive for both IgG and IgM antibodies. Overall seroprevalence in goats was 14.32% (60/419). IgG antibodies were found in 50 (11.93%) while IgM antibodies were found in 12 (2.86 %) sheep. Two goats were positive for both IgG and IgM antibodies. No significant difference was observed according to seroprevalence in both species (Table I).

Analysis of the data collected from the questionnaires and interviews revealed that likelihood of infection increased with age in both species. Female animals of both species were more likely to be infected as compared to males (OR 1.85 and 1.70 in sheep and goats respectively). Further statistically significant risk factors in sheep were poor hygienic conditions (OR = 4.91, p<0.01), presence of cats (OR = 2.08, p<0.05), extensive
farming practice (OR = 2.19, p<0.05), flock size larger than 50 individuals (OR = 4.24, p<0.01) and pregnancy (OR = 2.50, P<0.01) (Table II). Similarly statistically significant risk factors in goats were poor hygienic condition (OR = 2.06, p<0.05), usage of outdoor water source (OR = 2.06, p<0.01), presence of cats (OR = 2.03, p<0.05), extensive farming practice (OR = 2.25, p<0.05) and flock sized larger than 30 (OR = 4.24, p<0.05) and 50 (OR = 6.82, p<0.01) individuals (Table III). No statistically significant breed wise difference of seroprevalence was observed in goats. However, seroprevalence was significantly high in salt range sheep as compared to other breeds of sheep (OR = 5.51, p<0.01).

**DISCUSSION**

Seroprevalence of *Toxoplasma*-specific IgG antibodies can be determined by various diagnostic
techniques in sheep and goats (Masala et al., 2003). There are incredible variations in behavior of different livestock species in different diagnostic tests. Moreover, there is no worldwide typical Toxoplasma gondii reference material available against which different diagnostic tests can be standardized. In the current study enzyme-linked immunosorbent assay was used which is appropriate for detection of Toxoplasma-specific IgG and IgM antibodies (Ghazaei, 2005; Vesco et al., 2007).

Results of our serologic study show that T. gondii infection is widely present in sheep and goats in northern Punjab, Pakistan. Previously, T. gondii infection in sheep is also reported from Egypt, Zimbabwe, Serbia, Brazil, South Africa and Saudi Arabia (Marompot and Botros, 1972; Hove et al., 2005; Klun et al., 2006; Romanelli et al., 2007; Samra et al., 2007; Sanad and Al-Ghabban, 2007). On the other hand prevalence in goats is also reported from Uganda, Thailand, Saudi Arabia, and Egypt (Bisson et al., 2000; Jittalpalapong et al., 2005; Sanad and Al-Ghabban, 2007; Barakat et al., 2009). Seroprevalence differs significantly in different areas due to difference in climate, hygienic conditions and farm managements (Zhao et al., 2011). Also it differs because of difference in serodiagnostic tests used (Vesco et al., 2007).

We found an increase in the prevalence of infection with age in both species. Increase in prevalence of the disease in older animals is due to exposure of animals to the risk factors for longer period of time than the younger ones (O’Donoghue

Table III.- Risk factors analysis of T. gondii infection in goats.

<table>
<thead>
<tr>
<th>Factors</th>
<th>Category</th>
<th>N</th>
<th>+ve</th>
<th>Prevalence % (95% C.I.)</th>
<th>Odds Ratio (95% C.I.)</th>
<th>p– value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>&lt;12</td>
<td>30</td>
<td>3</td>
<td>10.00 (03.46–25.62)</td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td></td>
<td>13–24</td>
<td>181</td>
<td>15</td>
<td>8.29 (5.09–13.23)</td>
<td>0.81 (0.22–3.00)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td></td>
<td>25–36</td>
<td>137</td>
<td>23</td>
<td>16.79 (11.46–23.93)</td>
<td>1.82 (0.51–6.49)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td></td>
<td>&gt;36</td>
<td>71</td>
<td>19</td>
<td>26.76 (17.85–38.05)</td>
<td>3.29 (0.89–12.11)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Sex</td>
<td>Male</td>
<td>153</td>
<td>16</td>
<td>10.46 (6.54–16.31)</td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>266</td>
<td>44</td>
<td>16.54 (12.56–21.47)</td>
<td>1.70 (0.92–3.13)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Breeds</td>
<td>Jattal</td>
<td>144</td>
<td>16</td>
<td>11.11 (06.96–17.29)</td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Beetal</td>
<td>161</td>
<td>22</td>
<td>13.66 (09.20–19.82)</td>
<td>1.27 (0.64–2.52)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td></td>
<td>Teddy</td>
<td>55</td>
<td>11</td>
<td>20.00 (11.55–32.36)</td>
<td>2.00 (0.86–4.64)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td></td>
<td>Beetal× Jattal</td>
<td>59</td>
<td>11</td>
<td>18.64 (10.74–30.37)</td>
<td>1.83 (0.79–4.23)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Hygienic condition</td>
<td>High</td>
<td>138</td>
<td>14</td>
<td>10.14 (06.14–16.30)</td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Moderate</td>
<td>159</td>
<td>23</td>
<td>14.47 (09.84–20.78)</td>
<td>1.50 (0.74–3.04)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td></td>
<td>Low</td>
<td>122</td>
<td>23</td>
<td>18.85 (12.90–26.70)</td>
<td>2.06 (1.01–4.21)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Source of Water</td>
<td>Indoor</td>
<td>259</td>
<td>28</td>
<td>10.81 (07.59–15.18)</td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Outdoor</td>
<td>160</td>
<td>32</td>
<td>20.00 (14.54–26.87)</td>
<td>2.06 (1.18–3.58)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Presence of cats</td>
<td>No</td>
<td>142</td>
<td>13</td>
<td>9.15 (05.42–15.03)</td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>277</td>
<td>47</td>
<td>16.97 (13.01–21.84)</td>
<td>2.03 (1.06–3.89)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Management</td>
<td>Intensive</td>
<td>93</td>
<td>8</td>
<td>8.60 (4.42–16.06)</td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Semi-intensive</td>
<td>143</td>
<td>20</td>
<td>13.99 (9.24–20.62)</td>
<td>1.73 (0.73–4.10)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td></td>
<td>Extensive</td>
<td>183</td>
<td>32</td>
<td>17.49 (12.67–23.65)</td>
<td>2.25 (0.99–5.11)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Flock Size</td>
<td>&lt;10</td>
<td>42</td>
<td>2</td>
<td>4.76 (1.31–15.79)</td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td></td>
<td>11–30</td>
<td>175</td>
<td>18</td>
<td>10.29 (6.61–15.68)</td>
<td>2.29 (0.51–10.29)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td></td>
<td>31–50</td>
<td>143</td>
<td>25</td>
<td>17.48 (12.13–24.53)</td>
<td>4.24 (0.96–18.69)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td></td>
<td>&gt;50</td>
<td>59</td>
<td>15</td>
<td>25.42 (16.05–37.79)</td>
<td>6.82 (1.47–31.69)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Reproductive Status (Females)</td>
<td>Non-Pregnant*</td>
<td>89</td>
<td>16</td>
<td>17.98 (11.38–27.23)</td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pregnant</td>
<td>72</td>
<td>16</td>
<td>22.22 (14.17–33.09)</td>
<td>1.30 (0.60–2.83)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td></td>
<td>Lactating</td>
<td>105</td>
<td>12</td>
<td>11.43 (06.66–18.92)</td>
<td>0.59 (0.26–1.32)</td>
<td>&gt;0.05</td>
</tr>
</tbody>
</table>

*Non-pregnant = neither pregnant nor lactating
et al., 1987; Puije et al., 2000). Similar findings in sheep and goats are also reported by Vesco et al. (2007), Lashari and Tasawar (2010) and Tasawar et al. (2011). It is also reported previously that susceptibility to infection from protozoan parasites is found greater in female animals as compared to males (Alexander and Stinson, 1988). We also observed higher prevalence of T. gondii infection in female animals which may be explained by the fact that immunity in females is reduced by various factors such as pregnancy, nutrition and lactation (Martin, 2000; Kelly et al., 2001). We also found significant higher seroprevalence in pregnant sheep (OR = 2.50, P <0.01) and non-significant higher seroprevalence in pregnant goats (OR = 1.30, P>0.05). Higher prevalence of toxoplasmosis in female goats is also reported by Tasawar et al. (2011). Current study also revealed unusual high prevalence in salt range sheep as compared to other breeds of sheep. However, it was observed that salt range sheep in the region was mostly overcrowded and were managed under extensively farming practice which may increase the risk of infection.

No statistically significant difference of seroprevalence was observed in sheep and goats from different districts which suggest uniformity of the climate and management practices in the study area. Analysis of other risk factors showed outdoor water source was a putative risk factor for goats but not for sheep (Tables II and III). This may be explained by the facts that goats are mostly infected by drinking contaminated water. Chances of getting infection through food are low as they usually browse the leaves which are far from the ground. On the other hand sheep graze close to the ground and have better chances of getting infection via both food and water (Waldeland, 1976).

Presence of cats also significantly contributed in increasing the likelihood of infection in both sheep and goats in present study. Cats are the definitive hosts of the parasite and play a vital role in infecting other animals by shedding oocysts in the environment (Lopes et al., 2010). A study from Poland reported that the presence of free-roaming cats is an important risk factor for the transmission of the infection in goats (Neto et al., 2008). Similar findings have also been reported in other studies in livestock animals (Puije et al., 2000; Chaudary et al., 2006). Lopes et al., 2010; Ahmad and Qayyum, 2014.

Seroprevalence was also higher in animals kept under poor hygienic conditions. Poor hygienic condition may favor the food and water to be contaminated with cat faeces, hence increase the likelihood of infection in livestock animals (Hove et al., 2005). Current study also found higher seroprevalence in livestock animals raised in extensive and semi-intensive management system. Extensive management in sheep and goats presents greater risk of T. gondii infection. Moreover, the use of bulk feed or pasture also poses threat of getting toxoplasmosis. Both practices render animals to come closer in contact with oocysts shed by wild and domestic felids. As compared to extensive or semi-intensive management, animal raised intensively are usually caged and get little chance to ingest oocysts contaminated food and water (Anderlini et al., 2011). Similar findings have been reported in Ghanaian and Brazilian sheep raised extensively (Puije et al., 2000; Lopes et al., 2010). Increase in prevalence of toxoplasmosis in extensive management is also found more recently in sheep and goats from northeastern China (Wang et al., 2011).

Current study also found significant increase in seroprevalence in animals which were kept in larger flocks. Although toxoplasmosis cannot be transferred from animal to animal however, higher seroprevalence in larger flock may be explained by the fact that the larger flock sampled in the study were mostly managed under semi-intensive or extensive system of management which increases the risk of getting toxoplasmosis as compared to intensive system of management. Similar findings have been reported in cattle and sheep in Serbia (Klun et al., 2006). More recently, increase in prevalence of T. gondii infection with flock size is also reported in a Brazilian study (Anderlini et al., 2011).

In summary, the results of current study confirm the presence of Toxoplasma gondii infection in sheep and goats in Pothwar region of Punjab. This verification of seroprevalence in sheep and goats indicated that toxoplasmosis is widely present and may cause economic losses in livestock in the form of abortion and neonatal deaths. Further
investigations are needed to find out zoonotic impact of the parasite and to explore the economic losses due to this infection.

ACKNOWLEDGMENTS

We are grateful to Higher Education Commission of Pakistan for providing financial assistance to first author (Mr. Nisar Ahmad) under Indigenous PhD fellowship programme and farm managers at private animal farms and Livestock Production Research Institute, Kharimurat (LPRI), Fateh Jhang for providing experimental facilities.

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*(Received 13 August 2014, revised 27 August 2014)*