Short Communication

Seroprevalence of *Brucella abortus* in Yak, Zo and Cows in Gilgit and Nagar Districts of Gilgit-Baltistan, Pakistan

Waseem Abbas¹, Dildar Hussain Kalhoro¹*, Hasina Baloch¹, Muhammad Abubakar³, Muhammad Saleem Kalhoro¹, Neluma Wali², Mazhar Hussain Mangi¹, Azhar Ali Laghari⁴, Shahid Hussain Abro¹, Rani Wagan¹ and Mehkar Hussain⁵

¹Faculty of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University, Tandojam, Pakistan
²Department of Zoology, University of Gujrat, Gujrat, Pakistan
³National Veterinary Laboratories, Islamabad, Pakistan
⁴School of Environmental Science and Engineering, Tianjin University, Tianjin 300350, China
⁵Department of Microbiology, University of Karachi, Karachi, Pakistan

**ABSTRACT**

Brucellosis is one of the most infectious and zoonotic diseases of livestock. Experiments were conducted on serum of yak, zo and cow from Gilgit and Nagar districts of Gilgit Baltistan Pakistan. Seroprevalence and risk factors of brucellosis were recorded during this study. Confirmation of *Brucella abortus* in serum was carried out using Rose Bengal plate test (RBPT) and indirect enzyme linked immunosorbent assay (I-ELISA). Seroprevalence at district Gilgit was recorded 6.66% in cows and 6.66% in yak while it was 3.33% in zo. Seroprevalence at Nagar district was recorded as 43.33% in cows and 13.33% in yak while it was 10% in zo. The overall prevalence at district Gilgit was recorded 5.55% and it was 22.22% at Nagar district. The overall prevalence at summer season recorded was 20% and at winter season it was 7.77%. Highest positive cases were recorded in older animals as compared to young ones. Higher prevalence was observed in female animals as compared to males in both districts. However, all the 180 serum sample examined through RBPT and I-ELISA test suggested that high prevalence of brucellosis in yak, zo and cows in district Nagar as compared to Gilgit and leads to significant economic losses. This is the first study demonstrating the prevalence of an important pathogen in yak, zo and cow of Pakistan.

Livestock is the most important sector of agriculture in Pakistan. It accounts for 60.54 percent to the agriculture and about 11.22 percent to the GDP of Pakistan (Ejaz and Ahmad, 2017). The important prerequisite for efficient livestock production is to increase the productive efficiency of an animal to maintain the normal health. However, cow, buffalo, yak and zo in remote areas face serious problems in terms of insufficient nutrition, climatic conditions, improper management and various contagious diseases. Brucellosis is a distressing threat to the large ruminants in remote and less developed areas. Brucellosis is highly contagious zoonosis caused by nonspore forming, nonmotile, aerobic coccobacilli of *Brucella* species. Brucellosis causes infection in almost all domestic and wild animal species (cattle, buffalo, yak, zo, sheep, goat, camel and pig) (Saher et al., 2018). Gilgit-Baltistan has cultural diversity with a covering area of about 72,971 sq km. Gilgit Baltistan is bordered by China in north, India in south and Afghanistan in west. Pastoralists move with their livestock herds in search of water and pasture and these movements within national territories or cross country borders were not checked for a decades. Due to which trans-boundary animal diseases easily transmitted. Yak (*Bos grunniens*) is unique plateau specie that habitat in Tibetans for over 4,500 years (Harris and Loggers, 2004). The yak so called Tibetan cattle is mainly found in China, Mongolia, Russia, Nepal, India and Pakistan (Gilgit-Baltistan). Yak has high economic value as pack animal, source of milk, meat and fiber in Himalyan region of Nepal (Aryal and Paudel, 2017).
Pakistan is the third largest milk producing country in the world after India and China. The farming of large ruminants in Pakistan is threatened by the prevalence of many infectious and non-infectious diseases. Among the infectious disease, brucellosis is a major threat to large ruminants (Gul and Khan, 2007). Brucellosis is endemic in livestock and causes human disease in Africa, Asia, America, Egypt, Italy, Iran and Turkey (Pappas et al., 2006). Brucellosis is transmitted by direct contact with infected animal, contaminated environment and movement of infected animal being a key feature of disease transmission (Corbel, 2006). Brucellosis transmission to human occur directly via inoculation of the infected animal secretions and eating and drinking unpasteurized milk and meat products (Klous et al., 2016). In male it causes orchitis, epididymitis and permanent infertility (Poester et al., 2013). The clinical manifestation of disease is distinguished by abortion, retained placenta, weak calves, endometritis, decreased milk yield (Megersa et al., 2011). Risk factors such as overcrowding especially during confinement, stress due to weather change, lack of vaccination, poor management, parity, age, sex, breeding practices, geographic location and presence of susceptible wildlife contribute to the occurrence and spread of the disease (Asmare et al., 2013). In China seroprevalence in yak has ranged from 4.16% to 13.4% in Qinghai–Tibet plateau, Xianjiang and Sichuan provinces (Tiwari, 2019). Ali et al. (2017) reported 3 to 6.5% seroprevalence of bovine brucellosis from various areas of Pakistan. Relatively lower seroprevalence at animal level (5.0%) and herd level (6.5%) was reported in Uganda, that indicated local herd management is an important factor for the spread of Brucellosis (Matope et al., 2011). Several screening tests has been widely used around the developing countries to diagnose the brucellosis. Among the screening tests, RBPT and I-ELISA is commonly used as diagnostic tools to determine herd health status. I-ELISA has been proven to more sensitive and specific tool and may be used for brucellosis diagnosis tool than biochemical tests (Erdenebaatar et al., 2004). The current study was therefore, designed to know the prevalence of brucellosis in yak, zo and cows of Gilgit and Nagar districts of Gilgit Baltistan. Moreover, risk factors related to the prevalence of brucellosis were also investigated.

Materials and methods

The current investigation was carried out at District Gilgit and Nagar to record seroprevalence and risk factors of brucellosis in yak, zo and cows. To address the stated objectives, questionnaires was managed to select individual animals in study areas to gather information of vaccination, risk factors such as age, sex and seasons for brucellosis occurrence in the area. About 5 ml of blood samples, 60 from zo and 60 from cows were collected in a clot activator vacutainer. Serum was separated by centrifugation method and then analyzed by RBPT (UK company) and I-ELISA (IDEXX Serum x2 ELISA kit, BAT1132T, K421) for confirmation of disease as prescribed by OIE (2009).

The RBPT, ELISA results were summarized in excel 2013 and multivariate logistic regression was used to compare the seroprevalence and risk factors. P-value 0.05 was set level of significance.

Results

Table I shows the overall seroprevalence of Brucella abortus in yak, zo and cow in Gilgit and Nagar districts. Seroprevalence of B. abortus in district Gilgit was recorded as 2/30 (6.66%) in cows and 2/30 (6.66%) in Yak while it was 1/30 (3.33%) in Zo. The seroprevalence of B. abortus in Nagar district was recorded as 13/30 (43.33%) in cows and 4/30 (13.33%) in Yak while it was 3/30 (10%) in Zo (Table I). Results of both districts were compared statistically and indicated significant difference (P<0.05) in cows, yak, zo at district Nagar.

Table I. Overall seroprevalence of B. abortus in yak, zo and cow in Gilgit and Nagar districts.

<table>
<thead>
<tr>
<th>Area</th>
<th>Animal</th>
<th>Total samples tested</th>
<th>Sample positive</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gilgit</td>
<td>Cow</td>
<td>30</td>
<td>2 (6.66%)</td>
<td>0.806</td>
</tr>
<tr>
<td>Yak</td>
<td>30</td>
<td>2 (6.66%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zo</td>
<td>30</td>
<td>1 (3.33%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>90</td>
<td>5 (5.55%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nagar</td>
<td>Cow</td>
<td>30</td>
<td>13 (43.33%)</td>
<td>0.035</td>
</tr>
<tr>
<td>Yak</td>
<td>30</td>
<td>4 (13.33%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zo</td>
<td>30</td>
<td>3 (10%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>90</td>
<td>20 (22.22%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Age was also considered as major risk factor for seroprevalence of brucellosis in yak, zo and cow. Yak, zo and cow of 2-5 years were more seropositive than cow, yak and zo of <2 years. Yak, zo and cows of >5years were more seropositive than yak, zo and cows of 2-5 years (Table II). The statistical analysis indicated non-significant difference (P>0.05) among various age groups.

The overall prevalence of B. abortus in district Gilgit recorded was 5/90 (5.55%) while it was 20/90 (22.22%) in Nagar district (Table I). Results of both districts were compared statistically and indicated significant difference (P<0.05) in both districts.

Female cow, female yak and female zo were more seropositive than male yak, zo and cow (Table III). The statistical analysis showed non-significantly difference (P>0.05) for male and female.
Table II. Prevalence of *B. abortus* in yak, zo and cow in relation to the risk factor age.

<table>
<thead>
<tr>
<th>Age</th>
<th>Cow Male</th>
<th>Yak Male</th>
<th>Zo Male</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 2 yr</td>
<td>36</td>
<td>23</td>
<td>23</td>
<td>0.579</td>
</tr>
<tr>
<td>2-5 yr</td>
<td>8</td>
<td>7</td>
<td>7</td>
<td>0.72</td>
</tr>
<tr>
<td>&gt; 5 yr</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>0.72</td>
</tr>
</tbody>
</table>

Table III. Overall prevalence of *B. abortus* in yak, zo and cow in relation to the risk factor sex.

<table>
<thead>
<tr>
<th>Animal type</th>
<th>Sex</th>
<th>Sample positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yak</td>
<td>Male</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>0</td>
</tr>
<tr>
<td>Zo</td>
<td>Male</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>0</td>
</tr>
<tr>
<td>Cow</td>
<td>Male</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>10</td>
</tr>
</tbody>
</table>

Table IV. Seroprevalence of *B. abortus* in relation to the risk factor season.

<table>
<thead>
<tr>
<th>Seasons</th>
<th>Total sample</th>
<th>Positive</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Summer</td>
<td>90</td>
<td>18</td>
<td>0.043</td>
</tr>
<tr>
<td>Winter</td>
<td>90</td>
<td>7</td>
<td></td>
</tr>
</tbody>
</table>

In summer seasons more yak, zo and cow were seropositive than winter season (Table IV). The statistical analysis indicated significant difference (P<0.05) for both summer and winter seasons.

Discussion

Brucellosis is declared one of zoonotic disease of livestock and has great health and economic importance worldwide (Maadi *et al.*, 2013). In animals the economic losses from brucellosis are mainly because of clinical manifestation of abortion during last trimester of gestation, decreased milk yield and temporary infertility (Khan *et al.*, 2020). Brucellosis is evenly uncontrolled and endemic in Middle East, Asia, Latin America (Amaranth and Mantur, 2008). During present study total of 180 samples (60 each from Yak, Zo and cow) were collected from Gilgit and Nagar districts. Risk factors such as age, sex, seasonal effects and history were also collected from local farmers through questionnaires. Serum samples were first screened through RBPT, then confirmed by I-Elisa. Seroprevalence of *B. abortus* in district Gilgit was recorded as 2/30 (6.66%) in cows and 2/30 (6.66%) in Yak while it was 1/30 (3.33%) in Zo. The seroprevalence of *B. abortus* in Nagar district was recorded as 13/30 (43.33%) in cows and 4/30 (13.33%) in yak while it was 3/30 (10%) in zo. The low prevalence of *B. abortus* in yak and zo is due to the small herd size, natural breeding, minimal contact of yak and zo with local cows in grazing area and eating of medicinal plants over high pastures. On the other hand, minimum exotic infected animals are introduced in high pasture area of yak and zo. It is concluded that disease was prevalent in both districts however; relatively higher prevalence was observed in district Nagar. Results of both districts were compared statistically and indicated significant difference (P<0.05) in cows, yak, zo at district Nagar. The statistical analysis indicated non-significant difference (P>0.05) among various age groups. Results of both districts were compared statistically and indicated significant difference (P<0.05) at both districts. The statistical analysis showed non-significantly difference (P>0.05) for male and female. The statistical analysis indicated significant difference (P<0.05) for both summer and winter seasons. It is critical to understand the risk factors for a disease before developing and implementing a prevention or control program. The epidemiology of brucellosis is complex with multiple factors affecting the distribution of the disease. These include stocking density and herd size, age, sex and contact with wildlife (Megersa *et al.*, 2011). It has been reported that brucellosis is more common in female animals, although this may be influenced by the fact that females are kept for longer periods than majority of males and clinical signs of disease are also more apparent in females than males (Buzgan *et al.*, 2010). Al-Majali *et al.* (2009) reported mixed farming, particularly rearing sheep, goats along with cattle were major risk factor for transmission Brucella spp. In a retrospective study the seroprevalence of *B. abortus* in spring or summer found to be significantly higher than that in autumn or winter (Zeng, 2017). The spring or summer period, particularly the months of April and May, coincides with the peak calving time for yak. *Brucella* is primarily shed during abortions which typically occur during the third trimester of gestation (Anderson, 2007). This stage of the pregnancy coincides with the spring/summer period resulting in a greater opportunity for disease spread resulting in a higher incidence of disease. Traditional customs and methods of raising livestock also play an important role in disease transmission. Herders slaughtered old livestock animals and higher prevalence in aged animals lead to greater risk of brucellosis transmission (Boukary *et al.*, 2013). In Tibet due low temperature and high altitude allowed widen environmental survival of *Brucella* organisms potentially resulting in exposure of more yaks to the pathogen (Ostrowski, 2009).

Conclusion

It was concluded from present study that seroprevalence of *B. abortus* was significantly higher in Nagar as compared to Gilgit district. Low seroprevalence...
of B. abortus was recorded in yak and zo as compared to cows. Higher seroprevalence was recorded in older animals, female animals and in summer season.

Acknowledgement
The authors are highly thankful to the National Veterinary Laboratory, Islamabad, Pakistan.

Funding
The research was accomplished by utilizing routine working budget of National Veterinary Laboratory, Islamabad, Pakistan.

IRB approval
The research project was approved by the Directorate of Advanced Studies, Sindh Agriculture University, Tandojam, Pakistan.

Ethical statement
The study was approved by ethical committee vide No. Micr/58 of 2020 of Department of Microbiology, Sindh Agriculture University, Tandojam, Pakistan.

Statement of conflict of interest
There is no conflict of interest to declare.

References