Pakistan J. Zool., pp 1-4, 2023. **DOI**: https://dx.doi.org/10.17582/journal.pjz/20220426060412

Short Communication

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

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Article Information
Received 26 April 2022
Revised 20 November 2022
Accepted 15 December 2022
Available online 07 April 2023
(early access)

Authors' Contribution
WA, DHK, HB and SHA conceived and designed the experiments. RM, DHK, MA and MSK performed the experiments. NW, MHM, AAL, RW and MH analyzed the data. WA and DHK wrote the paper.

Key words
Brucellosis, Yak, Zo, ELISA, Gilgit Baltistan

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 and13.33% 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 plateu 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,

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0030-9923/2022/0001-0001 $ 9.00/0

2007). 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 along with infected animal, contaminated environment and movement of infected animal being a key feature of disease transmission (Corbel, 2006). Brucelosis transmission to human occur directly via inoculation of the infected animal secretions and eating and drinking unpasteurized mik 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 plateu, 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 at 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.

Area	Animal	Total samples tested	Sample positive	P value
Gilgit	Cow	30	2 (6.66%)	0.806
	Yak	30	2 (6.66%)	
	Zo	30	1 (3.33%)	
	Total	90	5 (5.55%)	
Nagar	Cow	30	13 (43.33%)	0.035
	Yak	30	4 (13.33%)	
	Zo	30	3 (10%)	
	Total	90	20 (22.22%)	

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.

Age	Cow		Yak		Zo	
	No	**Positive**	**No**	**Positive**	**No**	**Positive**
< 2 yr	1	1	2	0	2	0
2-5 yr	36	8	28	1	27	1
> 5 yr	23	7	32	2	32	3
P value		0.196		0.55		0.579

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

Animal type	Sex	Sample positive	P-value
Yak	Male	0	0.61
	Female	6	
Zo	Male	0	
	Female	4	
Cow	Male	3	
	Female	10	

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

Seasons	Total sample	Positive	P-value
Summer	90	18	0.043
Winter	90	7	

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*., 2011). 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 (Amarnath 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 questionaries. 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 thespring/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.

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