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THE SEROPREVALENCE OF *BRUCELLA ABORTUS* IN YAK, ZO AND COWS IN THREE DISTRICTS OF GILGIT-BALTITAN, PROVINCE

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An epidemiological cross sectional study was conducted from February to September 2017 to investigate seroprevalence of *Brucella abortus* in yak, Zo and cows in three districts (Ghizer, Skardu and Gilgit) of Gilgit-Baltistan Province. For that purpose a total of 160 serum samples (96 from yak & Zo & 64 from cattle) were collected through convenient sampling method. All sera were initially screened with Rose Bengal Plate test (RBPT). The results revealed that the seroprevalence of *Brucella abortus* in cows was 10.93% (7/64). It was an uncertainty that all seropositive sera were from Jersey cows of Government Dairy Farm Gilgit, kept for experimental purposes. It meant that the seroprevalence of *Brucella abortus* in that herd was found to be 87.5% (7/8). The local breeds of cow, Yak and Zo were free from *Brucella* infection in study area. After screening, RBPT positive samples were further tested with i-ELISA for confirmation. Out of 7 RBPT positive samples, 4 were found to be positive for the presence of antibodies. Hence the seroprevalence of *Brucella abortus* in exotic breeds of cow (Jersey) was 50% (4/8). The high prevalence of brucellosis in that herd may be due to poor management, lack of screening programs, using of infected bulls for breeding purpose and adapting of unhygienic veterinary practices etc. However further investigations are urgently needed.

The associated risk factors of *Brucella abortus* infection were identified through analyzing predesigned questionnaires in SPSS software version 16.0. The Chi square test results showed that fetal abortions having p-value P = 0.000 at (P < 0.05), OR; 18.500 at 95% CI; (2.299 to 148.838) and retained placenta with p-value P =0.009 at (P < 0.05), OR; 11.625 at 95% CI; (1.170 to 115.521) were found to be significantly associated with *Brucella abortus* infection. But the breed type (P = 0.013), animal age group (P =0.07), gender (P =0.351), disease history (P =0.04), and current health status (P =0.160) were not significantly associated with brucellosis at 95% CI: (P < 0.05). The current study revealed that the exotic breed of cattle might be the carrier of *Brucella* infection in disease free zone. Hence continuous surveillance of brucellosis in Government and private dairy farms may be recommended to reduce the chances of disease transmission in the selected region.

Keywords: Cow, brucellosis, antibodies, seroprevalence

Gilgit-Balitstan is a multicultural region with a covering area of about 27,971 km² (Hunzai 2013). It is in contact with China and Russian Free states at north, Afghanistan at west, India at east. Livestock movements across border and from down country is not being monitored for a decade. Due to which zoonotic and trans-boundary animal diseases may easily be transmitted. The yak so called Tibetan cattle (*Bos gruniens*) is a cloven footed furry bovid. It is mostly found in China, Mongolia, Nepal, Russia, India and Pakistan (Chitral & Gilgit-Baltistan). The housetrained strains of yak (*B. gruniens gruniens*) are very ample and closely linked with the livelihoods of village farmers (Shi et al. 2016). They are considered to be chief income source because people got meat, milk, fiber and skin within minimum inputs (Zeng et al. 2017).

The importance of yak research in high altitudes (Himalayan, Karakorum and Hindu Kush ranges) can be emphasized with following arguments. These areas are known for main yak breeding areas due to their suitable environment. Yak is considered as free income source for village farmers because they are remained outside in pastoral zones while grazing. On the other hand many of these ranges are declared as conservation parks. The wild life and yak of high altitudes may also attract tourists in the season. The link between village farmers, livestock experts, politicians, administrative sector and media should be reinvigorated (Jianlin et al. 2002).

The distribution of diversified wildlife favors the transmission of emerging and reemerging zoonosis in the world ecology. (Bengis et al. 2004). Brucellosis is a typical zoonosis caused by immotile, non-spore forming facultative coccobaccilli of *Brucella* species. Their natural hosts include cattle, buffalo, yak, sheep, goat and man (Manish et al. 2013). Brucellosis in cattle, buffalo and yak is mainly caused by *Brucella abortus* (Ali et al. 2013). The control and eradication of zoonotic brucellosis is not an easy job because of its intracellular persistent infection as an unknown illness and other political factors as well (Pappas et al. 2006). Brucellosis is endemic in yaks (*Bos gruniens*) and humans in Qinghai-Tibetan Plateau (QTP) due to poverty and bad hygiene. China has initiated a surveillance program in 2009, to control zoonosis effectively. As a result of which, its overall prevalence in livestock including yaks in Tibet was notified as 1.25% (Ma et al. 2016). On the ground, only 19% of people know about the symptoms of brucellosis in animals. People might be getting infected due to carelessness during animal handling. There is necessary to build a coordination medium among public health sector, veterinary professional's and wildlife authorities to provide better health schooling on brucellosis in different societies (Kansiime et al. 2014).

MATERIALS AND METHODS

A cross-sectional study was planned in 2017 to investigate the seroprevalence of *Brucella abortus* in yak, zo and cows in three districts of Gilgit-Baltistan, province (Ghizer, Skardu and Gilgit). A total of 160 (96 from yak/zo and 64 from cows) serum samples were collected through convenient sampling method from all mentioned species. Sera were initially screened by applying Rose Bengal Plat Test (RBPT). Then, the RBPT positive samples were subjected to i-ELISA for further confirmation. A predesigned questionnaire was also managed in parallel to identify different risk factors associated with the occurance of *Brucella abortus* in all animal species of specified area. Data pertaining to prevalence based on serum testing with RBPT, i-ELISA and field queries was analyzed on IBM, SPSS statistical software version 16.0. Chii Square test has been applied to check the relationship between test results and different risk factors.

RESULTS

An epidemiological cross sectional study was conducted to find out the seroprevalence of *Brucella abortus* in Yak, Zo and Cow in three districts of Gilgit-Baltistan. A total of 160 serum samples were collected and screened with Rose Bengal Plate Agglutination Test. The results showed that the seroprevalence of *Brucella abortus* in cows was 10.93% (7/64). All the seropositive samples were belonging to a single herd of Jersey cows kept by Livestock Department Gilgit-Baltistan for experimental purpose. The total population of that herd was 48. So the overall seroprevalence of brucellosis in that herd containing 48 cows was 87.5% (7/8). The local breeds of cow, Yak and Zo were negative for *Brucella* antibodies.

Table 4.1 (a) Table shows the overall results of RBPT

Animal Specie	Samples tested with RBPT	RBPT Positive	% age of Prevalence
Cow	64	7	10.94%
Yak and Zo	96	0	0
Total	160	7	4.4%

Table 4.1 (b) Table shows the district wise results of RBPT

S No	Area	Animal breeds	No of Samples tested with RBPT	RBPT Positive	% age of Prevalence
1	Govt. Dairy Farm Konodass Gilgit	Jersy cows	08	07	87.5%
2	District Ghizer	Yak and Zo	54	00	00
		Cows	33	00	00
3	District Skardu	Yak and Zo	42	00	00
		Cows	23	00	00
	Total		160	7	

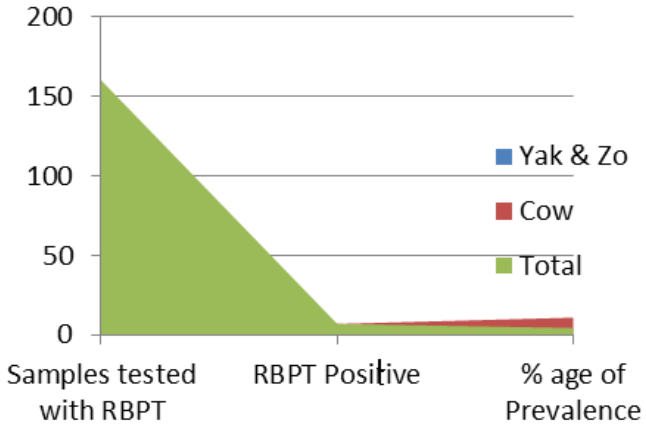
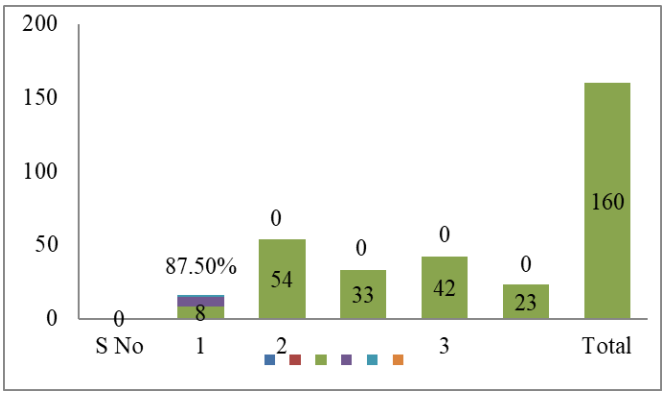


Figure 4.1 a) The above bar charts show the results of RBPT



(1 = Gilgit, 2 = Ghizer, 3 = Skardu)

Figure 4.1 b) The above bar charts shows the district wise results of RBPT

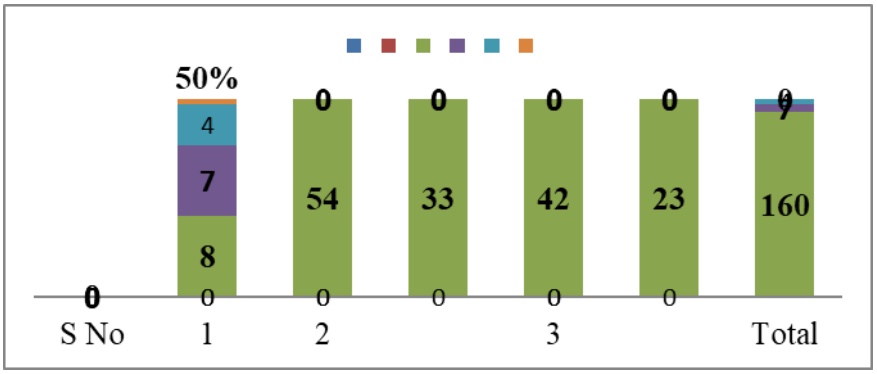
The Results of ELISA Test

After screening, RBPT positive samples were further tested by indirect ELISA for confirmation. According to which out of 7 RBPT positive sera, 4 were found to be seropositive to *Brucella abortus* infection. Hence the seroprevalence of *Brucella abortus* in exotic breeds of cow (Jersey) was got 50% (4/8).

The field data was analyzed through SPSS version 16. The dependent variable was "ELISA" as gold standard test while independent variables were animal breed, age, sex, shed type, current health status, disease history, history of abortion and history of retained placenta. Chi square test was conducted to interpret the data. The Chi square value, P-Value and odd ratio were

Table 4.2 The following table shows ELISA results of RBPT positive samples with percentage prevalence of *Brucela abortus* in cattle, yak and Zo.

S No	Area	Animal breeds	No of samples	RBPT Positive samples tested with ELISA	ELISA positive	% age of Prevalence
1	Govt. Dairy Farm Konodass Gilgit	Jersy cows	08	7	4	50%
2	District Ghizer	Yak and Zo	54	00	00	00
		Cows	33	00	00	00
3	District Skardu	Yak and Zo	42	00	00	00
		Cows	23	00	00	00
	Total		160	7	4	--



(1 = Gilgit, 2 = Ghizer, 3 = Skardu)

Figure 4.2 The bar charts shows ELISA results of RBPT positive samples

obtained to check the association of ELISA positive groups and different risk factors. A P-Value ($P < 0.05$) and an odd ratio having minimum range (MR < 1.000) were considered as significant. Different bar charts were obtained to elaborate test results more conveniently. From chi square table it can be concluded that the history of abortion (O.R 18.500 at 95% CI; range between 2.299 and 148.838) and the history of retained placenta (O.R 11.625 at 95% CI; range between 1.170 and 115.521) were significantly associated with the seroprevalence of *Brucella abortus* in study area. On the other hand breed type, animal sex, age group, disease history, animal shed type and animal's current health status were not significantly associated with the seroprevalence of *Brucella abortus* as their odd ratios were smaller than 1.000 at 95% confidence interval.

DISCUSSION

A cross sectional study was designed to investigate seroprevalence of *Brucella abortus* in Yak, Zo and cows in three districts of Gilgit-Baltistan. For that purpose a total number of 160 serum samples (96 from Yak & Zo, 64 from cows) were collected through convenient sampling method. All sera were initially screened by Rose Bengal Plat Agglutination Test (RBPT). The test has still used in serological investigations for the detection of *Brucella* antibodies in many parts of the world. An epidemiological investigation was conducted in Pantnagar, India to evaluate effectiveness of RBPT in the field for correct screening of *Brucella* infections. It was found that the RBPT having κ, 0.8597 would be reliable for detecting antibodies of brucellosis in field level over endemic areas of bovine brucellosis (Kushwaha et al. 2016).

As a result of initial screening, 7 sera of cows out of 64 were seropositive to *Brucella abortus.* But the detail of same results

indicated that all (7/64) seropositive serum samples were from a single herd of Jersey cows kept in Government Dairy Farm, Gilgit. The local breeds of cow, Yak and Zo were found free from brucellosis. It meant that the herd was severely infected with brucellosis. It is an alarming situation for the Department because the many livestock farmers are being provided breeding bull for breed improvement in the area. It may transfer *Brucella abortus* to disease free animals. However further investigations may be recommend to cope into the threatening condition.

After initial screening, RBPT positive samples were further tested with ELISA for confirmation. Out of 07 RBPT positive samples of that herd, 04 sera were seropositive to *Brucella abortus* infection. The results of RBPT and ELISA were slightly different with a variation of three samples. Because RBPT can better detect IgM (acute infection) and ELISA can better detect IgG (chronic infection or vaccinated animals) in sera of individuals. The variation of three samples may be due to the presence of three acute infections of *Brucella abortus* in the herd which could not be detected by ELISA. The above argument can be proved by a study conducted in Belgaum Institute of Medical Sciences (BIMS) Hospital, India from October 2007 to December 2008, to evaluate the effectiveness of ELISA test in detecting *Brucella* antibodies. It concluded that the sensitivity of ELISA test in acute cases was 28% at $P < 0.02$ and in chronic cases was 55% at $P < 0.01$ (Mantur et al. 2010). A study conducted in Tamil Nadu Veterinary and Animal Sciences University, revealed that both RBPT and ELISA were evaluated in an organized dairy and showed that the RBPT and I-ELISA may be combined to investigate brucellosis in the areas of low prevalence (Rekha et al. 2013). Another study was planned on 'Attwood' Veterinary Research Station, Australia. In which *Brucella*-specific antibodies were evaluated with respect to their efficiency in serological tests for bovine brucellosis. In which IgM reacted more efficiently than IgG_1 and IgG_2 in both the Rose Bengal plate test and serum agglutination test (Allan et al. 1976).

The ELISA results revealed that the seroprevalence of *Brucella abortus* among Jersey cows was 50% (4/8). As already mentioned that all seropositive cases were from a single herd where exotic breeds were kept for experimental purposes. Local cows, Yak and Zo were already excluded during initial screening due to showing negative results. It meant that local cows, Yak and Zo were free from bovine brucellosis in study area. That disease free status in local breeds may be due to the small herd size, natural breeding, isolate grazing area, high immunity and eating of medicinal plants over high pastures. On the other hand infected animals are not yet introduced in study areas.The same results were obtained in a study conducted in Mustang, Myagdi and Solukhumbu districts of Nepal. The objectives were to investigate seroprevalence of brucellosis and its associated risk factors in yak. About 678 serum samples were analyzed through ELISA. However, all serum samples were found negative to the *Brucella abortu*s antibodies. But the recorded cases of fetal abortions and retained placenta in Yak may be due to unknown reasons in Mustang (Aryal and Paudel 2014). Another study was conducted in Tibet, China in 2015. About 1523 serum samples of yak owned by 181 herders were tested with RBPT and C-ELISA. The individual prevalence of brucellosis in yak was found to be 2.8% at 95% CI. And the herd prevalence was 18.2% at 95% CI. The poverty, bad hygiene and large herd size were considered as facilitating agent for the transmission of brucellosis (Zeng et al. 2017). In the current study the Rose Bengal Plat Test (RBPT) was used as screening test whereas i-ELISA was chosen as a gold standard test. An epidemiological study conducted in Belfast, Northern Ireland to evaluate the performance of Serum Agglutination Test (SAT), Rose Bengal Plat Test (RBPT) and i-ELISA. About 19935 serum samples tested. It was found that the relative sensitivity was highest in i-ELISA (67.9%) and that of RBPT (78.1%) (Abernethy et al. 2012).

The field data was analyzed through SPSS version 16. The dependent variable was “ELISA test” while independent variables

were animal breed, age, sex, shed type, current health status, disease history, history of abortion, history of retained placenta and RBPT. Interpretation of data was made through Chi square test. The P-Value and odd ratio were used to check, either the results were significant or not. A P-Value ($P < 0.05$) and odd ratio more than 1.000 at 95% confidence interval were considered as significant.

On the basis of statistical results different risk factors were analyzed. The association between the seroprevalence of *Brucella abortus* and breed type has been interpreted according to Chi square test. The Chi square value was found to be 6.154 and that of P-value 0.013. The obtained p-value shows the association between breed type and seroprevalence of *Brucella abortus* at ($P < 0.05$). But the value of odd ratio (0.385 at 95% CI; range between 0.315 and 0.469) showed that the association was not significant. A study conducted in Sudan, to find out associated risk factors with brucellosis. It was conclude that the herd type, breed type, veterinary services, vaccination, awareness, bull share, age and gender ($P < 0.05$) were found positively associated with bovine brucellosis (Elmahi 2016).

The relationship of seroprevalence of *Brucella abortus* with animal sex was also analyzed. The obtained P-Value, 0.351 (greater than 0.05) and odd ratio 0.821 at 95% CI; range between 0.762 and 0.883 revealed that animal sex is not associated with *Brucella abortus* infection in study area. The publishing data from different studies conducted at international level supported the above argument. In a cross sectional study different risk factors associated with the seroprevalence of bovine brucellosis in cattle were investigated in different areas of Zimbabwe. About 1440 sera from 203 herds of cattle were tested with RBPT and c-ELISA. According to statistical calculations the overall individual based seroprevalence was 5.6% at 95% (CI). The seropositivity was free from sexual category. Cows with history of abortions were more seropositive 7.9% at 95% CI than cows with no history of abortion in study area (Matope et al. 2011).

The relationship of seropositivity of brucellosis with animal age group was assessed through Chi square test. According to the obtained P-Value 0.07 (greater than 0.05) and odd ratio 0.545 at 95% CI; range between 0.472 and 0.629 revealed that the result was not significant. So we can say that the seropositivity of bovine brucellosis is independent of animal age groups. The disease may affect equally all age groups. According to a cross sectional study the seroprevalence of *Brucella abortus* had an increasing trend with animal age. It showed that adult cattle (>6 years of age) were showing the higher seroprevalence (09.1%) than the animals of age below 6 years, showed 07.5%. But the variation was not statistically considerable (Gomo et al. 2012).

In current study the data relevant to animal disease history was analyzed to assess its possible association with seroprevalence of brucellosis. The obtained P-Value, 0.048 (smaller than 0.05) showed a clear association but the odd ratio 0.500 at 95% CI; range between 0.427 and 0.585 indicated that the results were statistically not significant.

The association between the seroprevalence of *Brucella abortus* and fetal abortions was investigated. According to statistical analysis the obtained P-Value (0.000) was much lesser than 0.05. On the other hand the values of odd ratio (18.500 at 95% CI; range between 2.299 and 148.838) clearly indicated that the results are highly significant. It meant that animals with the history of fetal abortions were interlinked with *Brucella abortus* infections in study areas. In a cross sectional study carried out in traditional livestock herds of Zambia to measure the role of *Brucella abortus* infection in fetal abortions. About 914 cattle sera from 124 herds were tested. Among positive cases about 12.6% cows had aborted their fetuses previously. The statistical analysis indicated a clear association between seroprevalence of *Brucella abortus* and fetal abortions in cattle in study population (Muma et al. 2007). In a cross sectional study, about 973 serum

samples were tested with ELISA in Punjab, India to investigate the seroprevalence of brucellosis and associated risk factors. The results showed that about 12.09% sera were seropositive to *Brucella* infection. The animals with history of abortions were showed high antibody reactions under chi square test as 24.50, $p < 0.001$ (33.87%) than those animals without abortion history (11.63%) (Kumar et al. 2005).

The retention of placenta may also be interlinked with the *Brucella abortus* infection. According to Chi square table the obtained P-Value 0.009, was much smaller than 0.05. Similarly odd ratio was obtained as 11.625 at 95% CI; range between 1.170 and 115.521. Both types of values were indicated that the association was highly significant. It is recommended that all veterinary professionals and workers should take biosafety measures during handling of animals with the history of fetal abortions and retained placenta. In a study conducted in Bangladesh in 2012 – 13, to assess different risk factors associated with the seroprevalence of brucellosis. According to which the seroprevalence of *Brucella abortus* was 10% in animals with fetal abortions, 4% in cows with retained placenta and 2.85% in cows with the complaint of repeat breeding (Islam et al. 2014). The prevalence, risk factors and economic impact of bovine brucellosis were assessed in cattle herds of Khartoum State, Sudan. Out of 1286 sera tested, 332 samples were positive to RBPT (26%). All RBPT positive samples were exposed to c-Elisa for further confirmation. The seroprevalence of bovine brucellosis was noted as 25.80%. Out of many risk factors breed type, veterinary services, vaccination, lack of awareness; bull share, age, abortion history and gender ($P < 0.05$) were found to be associated with bovine brucellosis (Elmahi 2016).

According to the statistical results the seropositivity of bovine brucellosis was independent of animals' current health status. The P-value 0.160 was much greater than P-Value 0.05. And the values of odd ratio 0.962 at 95% CI; range between 0.932 and 0.992, clearly showed that the results were not significant.

The comparison of ELISA figures and RBPT figures has been made through Chii square test. According to which the result was highly significant because the obtained P-Value 0.000 was lesser than standard P-Value at ($P < 0.05$). It meant that Rose Bengal Plate Test is still considered as reliable rapid test in diagnostic laboratories. A similar type of study was planned in the Ivory Coast in 2005 – 2009 to evaluate the performance of RBPT and i-ELISA tests. For this purpose, about 995 cattle sera were analyzed. A Bayesian technique was used to evaluate their sensitivity and specificity. The correlation adjusted sensitivity of i-ELISA and RBPT were 96.1% and 54.9%, respectively. High correlation adjusted specificity of i-ELISA and RBPT were 95.0% and 97.7%, respectively. The factual seroprevalence of brucellosis was assessed to be 04.6%. The results climax the significance of using both tests in combination should be as part of any Brucellosis Control Program (Sanogo et al. 2013)

The prevalence, risk factors and economic impact of brucellosis were assessed in cattle herds of Khartoum State, Sudan. Out of 1286 sera tested, 332 samples were positive to RBPT (26%). All RBPT positive samples were exposed to c-Elisa for further confirmation. The seroprevalence of bovine brucellosis was noted as 25.80%. A total of 14 risk factors i.e age, sex, herd size, geography, history of abortion , history of vaccination, mixed farming, type breed, mixed age, calves bar, breeding methods (natural ,artificial), presences of veterinary services, awareness and water supply were probed. The outcomes of the uni-variate chi square test shown that 11 risk factors (locality, herd type, breed, veterinary services, vaccination, awareness, bull share, water source, housing , age , and gender ($P < 0.05$) were found to be associated with bovine brucellosis. (Elmahi 2016).

The data of breeding style, water resource, quarantine measures, grazing/feeding style and vaccination record for assessing its possible association with the seroprevalence of Brucella abortus. Its mono-variable nature becomes hurdle to analyze under chi square test. Because there

is going lack of continuous surveillance, quarantine measures, regular vaccinations, schooling of local farmers regarding emerging and reemerging disease patterns and adapting of un-hygienic conditions in remote areas may transmit zoonotic diseases like brucellosis. However those mentioned factors would not be neglected in adapting brucellosis control strategies.

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