Human & Veterinary Medicine
International Journal of the Bioflux Society HVM

OPEN ACCESS
Research Article

Seroprevalence and potential risk factors associated with brucellosis in the Desert Thal of Pakistan

[1]Aamir Shehzad, [1]Fedik Abdul Rantam, [3]Awais Masud and [4]Shahbaz Ahmed

[1] Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, Indonesia; [3]Disease Diagnostic Laboratory, Livestock & Dairy Development Department, Mianwali, Punjab Pakistan; [4] LES Rakh Ghulama Livestock & Dairy Development Department Bhakkar, Punjab, Pakistan.

Abstract. Brucellosis is one of the widespread zoonosis considered as an emerging hazard for humans and animals. The current study is designed to investigate not only the prevalence but also the potential risks associated with brucellosis to the health of humans and domestic animals in desert Thal of Pakistan. A total of 1019 serum samples comprising 957 (n=499 buffalo, n=244 cattle, n=214 camels) domestic animals of both sexes & all ages and 62 humans closely in contact with animals were collected. All bovine samples were tested through the Rose Bengal plate test (RBPT) and re-confirmed by the ELISA test, while human serum samples test was carried out with Febrile Antigen Kit. The overall seroprevalence of 3.5% was found in cattle and buffalos and 17.3% in humans. Sero-positive 77.8% of animals have a history of abortion while 60% miscarriages was found in the spouses of the infected personals. Direct contact with *brucella*-infected animals and the use of unpasteurized milk is the major source for transmission of brucellosis which causes reproductive disorders and other health problems in humans. Abortion is found to be statistically significant (p-value <0.05) in sero-positive animals for *brucella*. Bovine brucellosis is a zoonotic disease in the desert Thal of Pakistan with many risk factors maintaining and perpetuating its spread. Therefore a collaborative effort among veterinarians and public health authorities is needed for implementing control measures and for creating public health awareness about health problems associated with brucellosis in the desert Thal of Pakistan.

Key Words: Brucellosis, Abortion, Desert Thal, Prevalence, Pakistan.

Corresponding Author: F. A. Rantam; e-mail: fedik-a-r@fkh.unair.ac.id

Introduction

Brucellosis is a highly infectious zoonotic disease caused by a genus of Gram-negative bacteria *Brucella*. World Health Organization (WHO), Food and Agriculture Organization (FAO) and Office International des Epizootics (OIE) also consider Brucellosis as one of the most global zoonosis (Schelling et al 2003). The *Brucella* can affect almost all domestic animals except cat which has resistance against the disease. It can also cause infection in humans, other ruminants and marines as well. Brucellosis is renowned with different names including Malta Fever, Undulant Fever, Mediterranean Fever, Epizootic Abortion, Bang's Disease, and Contagious Abortion (Abubakar et al 2012; Junaidu et al 2008). Brucellosis is an important zoonosis that can cause significant reproductive losses in sexually mature animals (Forbes et al 1996; Wadood et al 2009). The disease mainly causes last trimester abortion, stillbirth, placentitis, orchitis, infertility and also with excretion of the organism in uterus discharges and milk (England et al 2004). The *Brucella* can penetrate the body through various routes like digestive tract, mucosal layers, intact skin and spread out the body through blood and lymphatics to the organs cause localized disease (Lapaque et al 2005). Brucellosis is an important disease not only in the perspective of animal and human health but also has socio-economic impacts in rural areas where income sources depend on livestock and dairy products (Maadi et al 2011). Livestock is a lifeline for 95% of the rural population of the developing world (Wadood et al 2009; Hoffmann, 1999). Animals are the main source for transmitting of brucellosis to the human through direct contact, by the use of raw milk, and other dairy products. Due to a lack of knowledge about clinical signs, ignorance and lack of interpretation may lead to the *Brucella* under-reporting (CDCP 2013; Dean et al 2012; Seimenis et al 2006).

Brucellosis is considered as an occupational disease for farm and livestock, slaughterhouse workers, veterinarians, meat inspectors and laboratory personals (Nimri, 2003). People consume dairy products, handle animals and animal carcasses in the area of endemic infection are at supreme risk for contracting brucellosis (Shaqra 2000; Fosgate et al 2002). Brucellosis has been eradicated in many part of developed countries but it is still not properly controlled in developing countries like Pakistan (McDermott et al 2013; Gul & Khan 2007). According to the World Health Organization (WHO), brucellosis is one of the most neglected diseases in Pakistan (WHO, 2012). There are many studies on seroprevalence of brucellosis in animals in different regions of Pakistan but the current study is the first

research in the desert Thal of Punjab, province in Pakistan and has uniqueness due to its investigative approach of seroprevalence in domestic animals and persons very close in contact to these animals. Moreover, the current study has been designed to explore potential risks associated with brucellosis in animals and humans in close contact to them. Furthermore this study find out the predisposing role that creates a sensitizing note for recognition of this biological agent in the future.

Materials and methods

The area of the study

The study was conducted in district Bhakkar, of desert Thal, at Pakistan located in province Punjab (Fig.1).The selected area has diverse ecological conditions such as minimum rainfall and very extreme weather conditions during the summer and winter seasons.

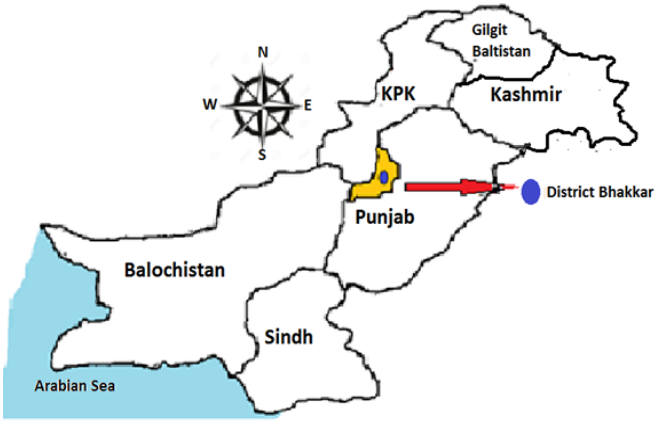

Fig. 1. Map of Pakistan showing the district Bhakkar

Samples and data collection

A total number of 1019 blood serum samples comprising 957 (n=499 Buffalo: n=244 cattle: n=214 camels) domestic animals of all sexes and ages, and 62 human workers and professionals of different categories [sweeper, gowalla (milkmen), bailder (men for watering the grasses), fodder cutter, artificial inseminators, veterinary assistants, veterinary doctors], in close contact with the domestic animals were collected. Blood sample approximately 10 ml was collected from the juggler vein of each animals under standard procedure (Alton et al 1988). The study population was selected using a pre-tested structured questionnaire and blood samples were obtained from the cattle, and buffalos. This questionnaire is used to know the various factors like histories of abortion, retention of placenta, dystocia, repeatability of selected livestock population. Another questionnaire was used to know the clinical signs and if any abnormality occur among the male humans and their spouses.

Before collection of data and blood samples, the purpose of the study and blood collection procedure were explained to the owner of the animals. Human blood samples were taken after getting consent from those in close contact with animals and livestock workers. The blood samples collection was done by a qualified medical personal. For this purpose, sterile syringes and needles were used to collect blood from cephalic veins of human workers of different categories closely in contact with the animals. All the blood samples were then immediately kept in an ice box and transported directly to the livestock disease diagnostic laboratories of district Mianwali and Bhakkar. All the blood samples were centrifuged at 1500 rpm for 10 minutes, and pure serum was separated and stored at - 20 0C for serological tests.

Serological Test

All serum samples of cattle, buffalo and camel were initially subjected to the Rose Bengal Plate Test (RBPT) antigen supplied by Veterinary Research Institute Lahore Pakistan. The RBPT was interoperated according to the described standard procedure of OIE (OIE, 2008). The RBPT is a sensitive serological test based on the principle of immunological reaction (agglutination) between the antibodies (agglutinin) produced due to the immune response of the body against Brucellosis and antigen of *Brucella*. The production of agglutination (clumping) indicates the presence of antibodies in serum and considered a positive test.

The human blood serum samples were tested with Febrile Antigen Kit (FAK) according to the standard advice of the manufacturer Bioactiva Diagnostica Germany. Serum samples of 25 µl were mixed with an equal volume of Rose Bengal antigen at glass plate, and glass plate was moved gently for 4 minutes. Only those serum samples considered positive in which agglutination occurred. Furthermore, the RBPT positive samples were reconfirmed through indirect Enzyme-Linked Immunosorbent Assay (iELISA). The iELISA was performed according to the advice of the Manufacturer ID.vet Luis Pasteur France in the provincial disease diagnostic laboratory at the directorate of animal disease reporting and surveillance system Lahore Punjab.

Result interpretation of iELISA

The optical density (OD) of the sample tested (S) was compared with OD of positive control (PC) and each sample was calculated in sample to positive ratios percentage (S/P %) as follows by using sample and control values:

S/P% = (OD samples - OD NC)/(OD PC - OD NC) x100

Samples with an S/P% less than or equal to 110% are considered negative, sample having S/P% greater than110% and less than 120% are considered doubtful and greater than or equal to 120% are considered positive. The OD was read and recorded at 450 nm. The test was considered valid only when the mean value of the positive control OD (OD PC) was greater than 0.350 (OD (PC)> 0.350) and the ratio of the mean values of the positive and negative controls (OD PC and OD NC) was greater than 3 (OD PC and OD NC > 3).

Statistical analysis

The data was analyzed by Chi-Square test using IBM SPSS Statics 21 software. The chi-square test was applied to check the association between various factors (abortion, retention of placenta, dystocia, repeatability), and seroprevalence. The level of significance set at $P<0.05$.

Results

All selected animals were properly visited and their owners were interviewed and then the blood samples were collected. A total number of 957 cattle, buffalos and camel samples respectively, were collected. The overall positive percentage of animals remained 7.52% (N=72) on the basis of RBPT. While

Table 1. Seropositivity of blood serum samples collected from animals and human

Species	Total Sample	RBPT Positive (%)	Tested with (FAK) Kit	ELISA Positive
Cattle	244	29 (11.9%)	0	17 (2.29%)
Buffalo	499	36 (7.2%)	0	9 (1.21%)
Camel	214	7 (3.27%)	0	0
Human	62	11 (17.74%)	11 (17.74%)	0
	1019	83 (8.14%)	11 (17.74%)	26 (2.71%)

Table 2. Abortion associated seroprevalence of *brucellosis*

Species	Positive Animals	Past Abortion History		Aborted animals %	*P*
		Yes	No		
Cattle	17	12	5	70.6	0.001
Buffalo	9	7	2	77.8	0.033
Total	26	19	7	73.1	

Table 3. Dystocia association seroprevalence of brucellosis

Species	Positive Animals	Past Dystocia History		Dystocia %
		Yes	No	
Cattle	17	0	17	0
Buffalo	9	0	9	0
Total	26	0	26	0

Table 4. Retention of Placenta association seroprevalence of brucellosis

Species	Positive Animals	Past Retention of Placenta History		Retention of Placenta %	*P*
		Yes	No		
Cattle	17	3	14	17.65	0.222
Buffalo	9	2	7	22.22	0.332
Total	26	5	21	19.23	

Table 5. Repeatability association seroprevalence of brucellosis

Species	Positive Animals	Past Repeating History		Repeating %	*P*
		Yes	No		
Cattle	17	2	15	11.76	0.333
Buffalo	9	2	7	22.22	0.332
Total	26	4	22	15.38	

based on ELISA it decreased to overall 3.50 % in cattle (2.29%) and buffalos (1.21%) but became negative in camels (Table 1). The RBPT depicted that 11.9% cattle found sero-positive for Brucellosis; buffalos 7.2%, and in camels 3.27%. It was found that 17.74% humans were sero-positive for brucellosis with RBPT and FAK (Table 1).

The study found a very high rate of abortion of 70.6% and 77.8% in cattle and buffalos respectively (Table 2) but no dystocia case found in any positive cattle and buffalo (Table 3) respectively. Retention of Placenta cases found 17.65% in cattle and buffalos 22.22%. In this study it was found that most of the animals

Table 6. *Brucella* positive human association with different health and other Factors

Total *Brucella* positive	17.7
Muscular Pain	18.2
Headache	27.3
Undulant (Fever) Temperature	9.1
Low blood Pressure	18.2
Abortion in Spouse (Miscarriage)	60
Arthritis (Skeletal complication)	9.1
Orchitis	9.1
Heart Diseases	18.2

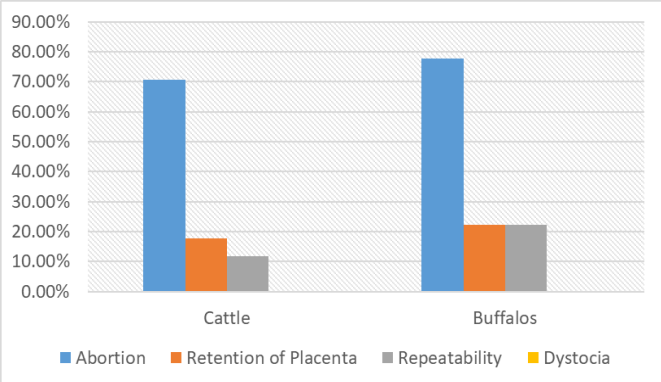

Fig. 2. Association between signs and seropositive animals

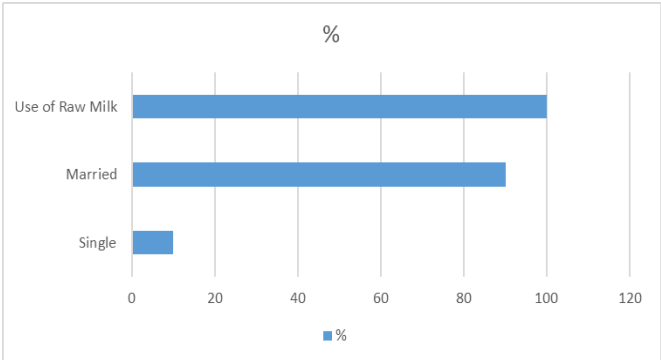

Fig. 3. Demographic of human

15.38% were repeating as in cattle 11.76% was found and in buffalos 22.22%. In the current study, there is found no cow bull, buff bull and camel bull positive for brucellosis.

A total number of 62 samples collected from humans which are very close to the animals, 17.7% were found to be positive for brucellosis. The most important thing is that in these positive samples the most affected people 45.45% were those who are gowallas (milkmen).

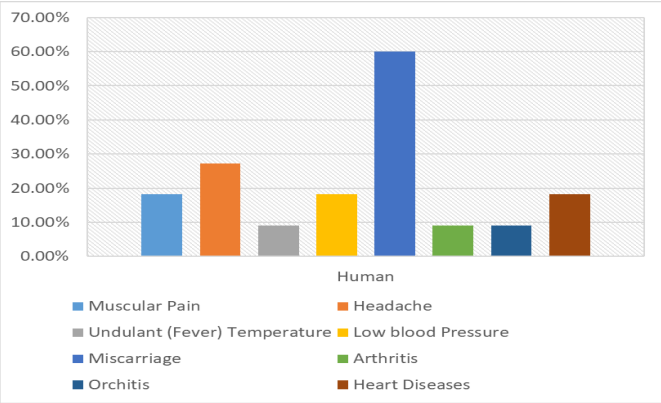

Fig. 4. Association between signs and seropositive Human

The different signs and symptoms found in sero-positive cases of brucellosis are muscular pain, low blood pressure, heart diseases (18.2%), headache (27.3%), undulant temperature, arthritis or skeletal complications, and orchitis 9.1%, respectively (Fig. 4). In the current study the major medical complication found is a high rate of 60% miscarriages in the wife's (spouses of the sero-positive male humans) (Fig. 4 and Table 6). All *Brucella*-positive people were found to be habitually using raw milk (Fig. 3). The association between abortion and sero-positive cattle and buffalos is found significant (P<.05) (Table2), while the associations between retention of placenta and seroprevalence of brucellosis, and repeatability of seroprevalence of brucellosis are insignificant (Table 4, 5).

All affected human use raw or unpasteurized milk. And found 90% people are married (Fig. 3).

Discussion

The current study is unique due to the first investigative approach of seroprevalence in domestic animals and humans very close in contact with them in the area of desert Thal. The findings of the present study show overall seroprevalence on the basis of RBPT in cattle, buffalos, and camels of 7.52% in which cattle have 11.9%, Buffalos 7.2% and camel 3.27%. The overall seroprevalence on the basis of RBPT in the current study is lower than 12.29% reported in a previous work on Food animals in Punjab Pakistan (Gul et al 2014). This low seroprevalence might be due to scattered animal populations in the area which could minimize the animal to animal direct contact and also possibly low due to extreme environmental conditions of the area. The current study area is mostly deserted, this may be the cause of low prevalence and this low seroprevalence support the published study of Ethiopia in which the author suggested seroprevalence rate high in riverine irrigated and cultivated areas (Bekele et al 2013). Based on literature, the results of seroprevalence in cattle are comparable with 10.18% and 10.32% showed in two different studies conducted in Pakistan (Gul et al 2014; Hussain et al 2008) and strongly agreed with 11.76% with an international study conducted in neighboring country India (Ghodasara et al 2010). The current study strongly agreed with the seroprevalence 7.40% in buffalos with RBPT which showed in a similar study, carried out in district Vadodara of India (Brahmabhatt et al 2009). Our study results of seroprevalence in buffalos are comparable with 6.6% of the previous study on the basis of MRT, conducted in nearby pothohar region of Pakistan and also agreed that cattle are more seroprevalent than buffalos (Ali et al 2013). The seroprevalence of brucellosis in current study 3.27% is almost the same and supported the study carried out in camels in which seroprevalence found 3.41% with RBPT (Shahzad et al 2017). The overall in cattle and buffalos seroprevalence of brucellosis 2.71 % is almost comparable with the research work at farm level in Punjab Pakistan, which showed overall seroprevalence 2.4% in cattle and buffalos (Hussain et al 2018) but the current result is slightly lower in comparison with the previous study carried out in northern Punjab Pakistan in which buffalo and cattle average seroprevalence was 3.13% (Abubakar et al 2010). This slight difference of prevalence may be due to variation in the environmental conditions of the area. The current study result of 1.21% comparable with another similar published work in which showed ELISA based seroprevalence of 1.6% in buffalos (Hussain et al 2018) and also the current result is comparatively slightly lower in the seroprevalence 2% showed in conducted work at District Rajanpur Pakistan (Ismail et al 2018). Previous work conducted in the northern region of Pakistan showed that seroprevalence of 3.13% in cattle (Abubakar et al 2010) which is higher than the 2.29% of the current study. This might be due to regional diversity in ecology. In the current study abortion history in cattle is strongly agreed with the previous study which showed 70.6% conducted in Quram Agency Pakistan but the abortion history showed 66.6% lower in buffalos. (Khan et al 2017). The current study reveals repeatability history 11.76% and 22.22% in cattle and buffalos respectively which is lower than the previous study 21.21% carried out in cattle at Assam India (Chettri et al 2015) but in buffalos, a previous study conducted in Indian Punjab showed 27.77% which is higher than the current study (Aulakh et al 2008).These high values might be due to the ecological effects or may be due to husbandry techniques used in the areas. The current study retention of placenta in cattle is 17.65% and in buffalo 22.22% but it is very higher than the study carried out in Bangladesh in which repeatability in cattle was 1.64% and retention of placenta is 7.41% (Amin et al 2004) but another study carried out in Indian Punjab it was 55.5% in cattle and 33.3% in buffalos (Aulakh et al 2008) This variation in results may be due to ecological conditions. In human brucellosis seroprevalence in males found 17.7% (n=11) which is very slightly lesser than the 21.7% reported in the study conducted on abattoir workers (Mukhtar & Kokab 2008). The seroprevalence of *Brucella* is higher in married people 90% which is higher than that was found in the study carried out in Samarqand Uzbekistan which was 47.2% (Earhart et al 2009). This difference may due to differences in ecological conditions. The study depicts that in *Brucella* sero-positive humans arthritis 9.1% it is lower than 46.6% which was found in retrospective analysis (Gotuzzo et al 1982) and this study in sero-positive humans found headache at 27.3% which is higher than 23% in a previous study conducted in Kuwait (Mousa et al 1988). Undulant (fever) temperature is found at 9.1% in the current study similar to the sign of undulant fever found in *Brucella* positive case in Korea with the same history of raw milk use as in current study all positive used raw milk (Park et al 2003) signs of *Brucella* positive in current study i.e. headache, fever, miscarriage, and skeletal complications also found comparable in the similar study conducted in Peshawar Pakistan

(Ahmad et al 2014). The main finding is that 60% of females (spouse) of *Brucella* positive male got miscarriage it is higher than the female infected with brucellosis of 43% found in the study of *Brucella* positive women in Saudi Arabia (Khan et al 2001). When statistical analysis SPSS was applied, the association between abortion with sero-positive cattle and buffalo is found to be significant which is in line with an already conducted study in Qurram Agency Pakistan (Khan et al 2017) but the association with placenta retention and repeatability is found insignificant.

Conclusion

The results of the current study indicate the seroprevalence of brucellosis in animals and humans of the desert Thal and also depict reproductive disorders in sero-positive animals. The association between sero-positive animals (cattle and buffalos) and abortion is found significant ($P < 0.05$). The habitual raw milk consumers and humans closely in contact with animals have *Brucella* seroprevalence of 17.74%. The sero-positive humans do not only have health problems like muscular pain, headache, low blood pressure, undulant fever, arthritis but also found to have a high miscarriage rate in their spouses.

The current study built an opinion that *Brucella* has a predisposing role in many diseases and other health complications.

Acknowledgments

Authors acknowledge the kind cooperation of animal owners and their acceptance for animals used in this study. The Laboratory staff of livestock, Disease Diagnostic Laboratories Mianwali and Bhakkar that provides expertise during the study are highly acknowledged especially Dr Gulam Murtaza (ADIO Bhakkar) and also the humans who accepted and allowed us for their sampling for the current study.

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Authors

•Aamir Shehzad, Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya 6011, Indonesia.

•Fedik Abdul Rantam, Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya 6011, Indonesia

•Awais Masud, Disease Diagnostic Laboratory, Livestock & Dairy Development Department, Mianwali 42200, Punjab Pakistan

•Shahbaz Ahmed, LES Rakh Ghulama Livestock & Dairy Development Department Bhakkar 30000 Punjab, Pakistan

Citation	Shehzad A, Rantam FA, Masud A, Ahmed S. Seroprevalence and potential risk factors associated with brucellosis in the Desert Thal of Pakistan. HVM Bioflux 2020;12(2):47-52.
Editor	Antonia Macarie
Received	6 February 2020
Accepted	24 February 2020
Published Online	9 April 2020
Funding	None reported
Conflicts/ Competing Interests	None reported