

Seropositivity of brucellosis in human and livestock in Tribal-Kurram Agency of Pakistan indicates cross circulation

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Abstract

Brucellosis is an endemic disease in Pakistan, yet an overall systemic surveillance data of the country is missing. This study aims to determine seroprevalence in domestic livestock population and high and low risk-associated humans of tribal Kurram Agency, Pakistan. A total of 567 random animal blood samples (148 cattle, 105 buffaloes, 154 sheep, and 160 goats), 197 human serum samples (n= 83 from low risk population n= 114 from high risk individuals) and ,in order to establish association between abortion and brucellosis, additional 395 animals with maximum of 4 weeks of foetal-birth history with or without abortion were initially screened by rose bengal plate test (RBPT) and further confirmed by indirect enzyme linked immunosorbant assay (I-ELISA). Our results indicated an overall seroprevalence of 4.73% in cattle, 4.76% in buffaloes, 1.95% in sheep, and 3.13% in goats. Interestingly, seroprevalence of brucellosis in males of cattle, sheep and goats was found higher as compared to females, while, it was found higher in male buffaloes as compared to female buffaloes. Furthermore, there was a statistically significant relationship between occurrence of brucellosis and abortion (Chi-square test, $p < 0.05$). The overall seropositivity in individuals at high risk (those with close and physical contacts with animals) was found 4.39% as compared to 1.20% seropositivity of low risk (people with no obvious close physical contacts with animals) general population. Interestingly, seroprevalence of human brucellosis was relatively higher in human females as compared to males. Overall, these results indicate a higher seroprevalence of brucellosis in human and their livestock animals.

Keywords: brucellosis, livestock, sero-prevalence, Kurram Agency Pakistan

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Introduction

Brucellosis is considered a global issue infecting most of the domestic animals. According to the Office International des Epizooties (OIE), brucellosis is the second leading zoonotic disease in the world after rabies (<http://www.oie.int/en/for-the-media/animal-diseases/animal-disease-information-summaries/>). Brucellosis is highly contagious diseases that predominantly infects livestock, but can also infect human beings reflecting threats to public health. Generally humans experience the diseases mainly due to ingestion of contaminated products, such as milk and meat, of infected animals or close physical contacts with infected animals or secretion. Improper disposal of the contaminated remainings such as aborted foetal contents may contaminate the environment that helps in dissemination of infection (Muma et al., 2006; Pappas et al., 2006). The disease is most frequently screened by various serological tests such as rose bengal test (RBPT), serum agglutination test (SAT), complement fixation test (CFT) and enzyme-linked immunosorbent assay (ELISA) in addition to classical approach of bacterial isolation and modern techniques of bacterial DNA detection by polymerase chain reaction.

Brucellosis is caused by brucella, a gram-negative coccobacillus, which can infect virtually all ruminants (Cloeckart et al., 2003; Cloeckart et al., 2001). Genetically, brucella seems to derive from a single variant of *B. melitensis*, however, for practical reasons they exist with different names, such as *B. abortus* that can infect cattle and buffalo, *B. ovis* mainly isolated from sheep, while *B. melitensis* can infect sheep and goats. These three species are also important zoonotic species and can infect human causing undulant fever or malta fever and hold its importance due to its zoonosis. In animals, brucellosis can cause a variety of diseases with clinical manifestation of decreased milk production and reproductive problems, such as abortion, retention of placenta and infertility. *B. abortus*, the causative agent of bovine brucellosis, is characterized by predominant late gestation abortion in cows, resulting in reproductive failure and consequently reduced milk production. Clinical manifestations of bovine brucellosis remain severe and prolong and depend mainly on age, reproductive and immunological status, route of infection, virulence and infectious dose of Brucella (Radostits et al., 2006; Xavier et al., 2009). *B. melitensis* infection greatly affects ewes as compared to rams and causes a late term abortion in pregnant animals. In ewes, abortion is less common as compared to birth of weak offspring. Retained placenta is a common problem of ewes in endemic flocks. Rams suffer from orchitis and epididymitis and in some cases polyarthritis in endemic flocks (Radostits et al., 2006).

Brucellosis remained relatively higher in the subcontinent, particularly, in Pakistan, India and Bangladesh. Despite, random reports of higher prevalence of brucellosis in Pakistan, concrete planning, control and surveillance strategy is totally missing (Abubakar et al., 2012). The prevalence of *B. abortus* in bovines has been reported in different regions of Pakistan as high as 3.25% (Ahmad et al.,

1990) and 4.4% (Naeem et al., 1990). Mukhtar & Kokab (Mukhtar and Kokab, 2008) demonstrated cross circulation of brucella to human by diagnosing seropositive employees working in abattoir in Lahore Pakistan. Additionally, other reports from Punjab province of Pakistan indicated an overall higher prevalence (5.05%) of brucellosis in cattle using a serum agglutination test (Ahmed and Munir, 1995), whereas lower prevalence of 1.46% and 1.93% in sheep and goat, respectively has documented (Nasir et al., 2000). The tribal region of Kurram Agency is located in the North-west of Pakistan sharing boarder with the eastern part of Afghanistan with an estimated human population of around 4.48 million. Livestock and agriculture is the main source of income and food of Kurram Agency inhabitants. Literature regarding seroprevalence of brucellosis in tribal areas of Pakistan is not available, and thus the current status of the disease is not known. The current study was thus designed to determine the seroprevalence of brucellosis in Kurram tribal Agency, of Pakistan. We report on the seroprevalence of brucellosis in livestock animals and human population of Kurram Agency of Pakistan.

Materials and Methods

Ethical approval: The study was approved by the ethical committee of the University of Hazara, Khyber Pakhtunkhwa Pakistan. Samples were taken after getting written consent from human.

Study population and sampling: Kurram Agency is located in the North-west of Pakistan (Latitude & Longitude (WGS84): 33° 49' 7" North, 70° 10' 24" East) with an area of approximately 3,380 km² and 4.48 million human population. This cross sectional study was carried between August 2014 and January 2015. A total of 567 blood samples were collected randomly from different animals (148 cattle, 105 buffaloes, 154 sheep and 160 goats). Additionally, 83 human blood samples were collected from general population with low risk for brucellosis (human population with no obvious close physical contact in their routine life) and 114 blood samples were collected from selected individuals of high risk population (those people that remained with a close or physical contact with animals such as animal handlers and butchers). Furthermore, in order to determine association between brucellosis and abortion additional 395 animal sera blood samples from females-animals (see Table 2) with a birth history of not older than 4 weeks with or without abortion were collected. Animals or human with a history of vaccination against brucellosis were excluded from the study. Serum was isolated from blood samples using standard protocols and stored 2-8 °C or frozen to -20 °C until the procedure was carried out.

Screening and confirmation for brucellosis: Subsequently, all serum samples were initially subjected to RBPT followed by confirmation by indirect ELISA (iELISA) of RBPT-positive samples. RBPT was performed as described earlier (Oie, 2008), while iELISA was performed as advised by the

manufacturer (Innovative diagnostics, Brucella multispecies ELISA kit). Rose bengal reagent was purchased from Veterinary Research Institute, Peshawar Pakistan. When mentioned, graph pad prism was used for statistical analysis to determine significant differences by using student T-test or chi square test. A p-value of ≤ 0.05 was considered statistically significant.

Results

A total of 567 blood serum samples (148 cattle, 105 buffaloes, 154 sheep and 160 goats) were initially screened by RBPT, which indicated that overall 6.08%, 8.57%, 3.25%, and 5.63% of cattle, buffaloes, sheep and goats, respectively, were seropositive. These presumably positive samples were then subjected for confirmation by iELISA and results indicated that 4.73% of cattle, 4.76% of buffaloes, 1.94% of sheep and 3.13% of goats were seropositive for brucellosis (Table 1). The locally developed RBPT reagent most probably reacted non-specifically. Results showed no significant differences in prevalence among different animals groups ($p > 0.05$) (Table 1).

Seroprevalence of brucellosis by iELISA was found higher in female's cattle, sheep and goats as compared to their males with exception of buffaloes, however, no statistical differences were found between male and female livestock population ($p > 0.05$) indicating that gender is not a significant contributing factor in the prevalence of brucellosis of livestock. Data analysis of seroprevalence of brucellosis in terms of age group wise revealed that all younger animals in cattle, buffaloes and sheep were found negative for brucellosis. Interestingly, for all animal groups, increase age was found associated with increase chances of positivity for brucellosis. This goes in parallel with oldest age group representing the highest seropositive cases of brucellosis in our study. Our results indicated that out of 54 samples of cattle tested from age group 25-48 months, the prevalence was recorded 1.85% both by RBPT and iELISA. However,

of 89 samples tested of cattle in age group > 48 months of age, seroprevalence was 8.99% by RBPT and 6.74% by iELISA. Although, prevalence was higher in age group > 48 , but no statistical difference was observed between the two age groups (Table 1). Out of 37 samples tested from age group of 25-48 months in buffaloes, the prevalence of brucellosis was 5.40% both by RBPT and iELISA. Out of 60 samples tested in buffaloes in age group > 48 , seroprevalence was 11.67% by RBPT and 8.33% by iELISA. In the case of sheep, out of 130 samples tested in age group < 24 months, the prevalence 0%, while of 24 samples tested from age group > 24 months, the prevalence was 12.5% by both RBPT and iELISA, which was significantly higher in age group < 24 months. In the case of goats, of 146 samples tested in age group < 24 months, 4.73% were found positive by RBPT and 2.05% by I-ELISA, while, of 14 samples tested from age group > 24 months, 14.30% were positive by both RBPT and iELISA, which was significantly higher.

Seroprevalence of Brucellosis in Human Population:

A total of 197 samples (83 low risk general population and 114 from high risk population with close contact with their companion animals) were collected and screened for brucellosis. Results indicated that one out of 83 (1.20%) in the low risk population and 5 of 114 (4.39%) of high risk individuals were positive for brucellosis. Statistically, there was no significant difference between the low risk and high risk associated individuals. Of the 83 human samples (70 males and 13 females) from low risk population no case was reported in female, while prevalence rate of 3.84% was detected in the male population of high risk and 5.56% in females (Table 1). Statistically, no significant difference was found between the male and female gender of human ($p > 0.05$). The prevalence of human brucellosis in the age group ≤ 30 years was found 3.57%, while it was 2.74% in age group of 31-50 years and 2.5% in age group ≥ 50 years. No significant differences were observed in seroprevalence of brucellosis between different age groups ($p > 0.05$).

Table 1 Seropositivity of serum samples collected from animals and human

Source	Total tested	Positive (%)	Gender		Age groups (months)		
			Male tested (%) ⁺	Female tested (%) ⁺	13-24 tested (%) ⁺	25-48 tested (%) ⁺	> 48 tested (%) ⁺
Cattle	148	7(4.7)	26 (3.8)	122 (4.9)	5(0)	54(1.8)	89(6.7)
Buffaloes	105	5 (4.8)	17 (5.8)	88 (4.5)	8(0)	37(5.4)	60(8.3)
Sheep	154	3 (1.9)	61(1.6)	93 (2.1)	130(0)	24(12.5)*	NA
Goats	160	5 (3.1)	72 (2.7)	88 (3.1)	146(2.0)	14(14.2)*	NA
Total	567	20 (3.53)	176 (2.8)	391(3.8)	289(1.0)	129 (6.2)	149 (7.3)
Humans (high risk)	114	5 (4.4)	70 (1.4)	13 (0.0)	NA	NA	NA
Humans (low risk)	83	1 (1.2)	78 (3.8)	36 (5.5)	NA	NA	NA

Above data shows seroprevalence of brucellosis determined by iELISA. Overall seroprevalence was significantly lower ($p < 0.001$; chi square test) and no significant differences in prevalence were observed among cattle, buffaloes, sheep and goat ($p > 0.05$). * indicates significant difference at p value 0.05. ⁺ indicates seropositivity in percentile of tested population type only. NA not applied

Prevalence of brucellosis in aborted /previously aborted animals: In order to investigate the role of brucellosis in abortion, we further analysed additional 395 animal sera of female only, which had a birth history with or without abortion. Of these 395 samples, 7 cattle, 9 buffaloes, 4 sheep and 3 goats had a repeated

history of abortion. Of note, we sampled only those animals with a birth of foetus not older than 4 weeks. Interestingly, of 7 cows that had history of abortion, 5 were seropositive by iELISA for brucellosis, while it was recorded 1 out of 115 (by iELISA) in cows with no history of abortion. Chi square analysis indicated a

significant association between abortion and seropositivity of brucellosis ($p < 0.0001$) (Table 2). In buffaloes, seroprevalence in animals with a history of abortion was 66.67%, both by RBPT and iELISA, while it was recorded 1.20% in buffaloes with no history of abortion indicating a significant relationship between abortion and brucellosis. In Sheep, seroprevalence in animals with a history of abortion was 50%, while in sheep with no history of abortion, none of the sample

could be declared seropositive indicating a significant higher prevalence of brucellosis in sheep with history of abortion (Table 2). In goats, seroprevalence in animals with a history of abortion was 66.67%, while it was 1.18% in goats with no history of abortion indicating a significantly higher seroprevalence of brucellosis in aborted goats. Overall, our results clearly indicate that there is a significant relationship between abortion and brucellosis.

Table 2 Abortion associated seroprevalence of brucellosis

Species	Past abortion History (yes/no)	No. of Samples Tested	No. of RBPT Positive Samples (%)	No. of I-ELISA Positive Samples (%)	p-value*
Cattle	Yes	7	5(71.43)	5 (71.43)	<0.001
	No	115	3 (2.61)	1 (0.87)	
Buffaloes	Yes	9	6 (66.67)	6 (66.67)	<0.001
	No	83	1 (1.20)	1(1.20)	
Sheep	Yes	4	2 (50.00)	2 (50.00)	<0.001
	No	89	0(0.00)	0(0.00)	
Goats	Yes	3	2 (66.67)	2 (66.67)	<0.001
	No	85	4 (4.71)	1 (1.18)	
Total		395	23(5.8)	18(4.5)	<0.001

Chi-square test was applied to analyze statistical difference

Discussion

This study was carried out to investigate the seroprevalence of brucellosis in domestic animals and human population at low or high risk of embracing infection. Brucellosis, due to its obvious potential of zoonosis, holds importance in addition to adversely affecting production capabilities of livestock animals. In this report, we comprehensively surveyed majority of domestic animals including humans in order to determine the occurrence of brucellosis in animals and human. Overall, our results indicated high seroprevalence in animals including those human that were at high risk of getting infection. Furthermore, abortion and brucellosis were significantly associated.

Brucellosis can infect a range of animals including human. In this current report, we mainly focused on the domestic animals as these are the main source of food and income for people living in Kurram Agency. Our results showed that the prevalence of brucellosis was almost similar in cattle and buffaloes (4.73% and 4.76%, respectively). This recorded observations are in agreement with other study conducted in Pakistan, which reported the seroprevalence in cattle and buffaloes 5.05% and 5.45% , respectively (Gul and Khan, 2007), however, they have used serum agglutination test, which might have produced some false positive results. In the current survey, seroprevalence in sheep and goats (1.95% and 3.13%, respectively) was found less than the larger animals. Other reports from other parts of Pakistan have reported slightly lower range such as 1.46% and 1.93% for sheep and goat, respectively, reported by Nasir *et al.* (Nasir *et al.*, 2000). In Sarab city of Iran, an overall 4.06% of 1500 livestock animals were found positive for brucellosis using RBPT test (Akbarmehr and Ghiyamirad, 2011). Similarly, in India 3-5% of animals were found infected with brucellosis using blood samples (Renukaradhya *et al.*, 2002), however,

milk ELISA screening indicated a significantly higher (18-20%) prevalence in the province of Punjab, India (Aulakh *et al.*, 2008). In china the prevalence of brucellosis recorded at an average rate of 0.06-0.09 between 1991-98 in animals, while 0.74% was recorded for human , however, since 1994 onward the human cases of brucellosis infection gradually increased from less than 500 cases to 1500-3000 case per year (Dequ *et al.*, 2002). Interestingly, our results indicated that the probability of getting brucellosis infection is heightened with increased age. This was shown by higher incidence rate in older animals particularly those with age more than 48 months. This is most probably due to the presence of brucella in the environment and with more exposure increase the chances of infection. It will be interesting to know the circulating strain and its frequency in the environment. Additionally, this higher level seroprevalence of brucellosis among the older animals may be attributed to the sexual development of animals with the propelling age (Amin *et al.*, 2005). The higher level of seroprevalence in adult stock has been endorsed in other studies as well (Abubakar *et al.*, 2012; Rahman *et al.*, 2006).

The prevalence of brucellosis among humans that remained in close contact with their domestic animals indicated around 4.5%, which was higher than 3.2% seroprevalence reported by Apan *et al.*, 2007 (Apan *et al.*, 2007). Females were found to be more seropositive (5.54%) as compared to males (3.85%). These findings are in line with those reported by Riaz *et al* from North Waziristan tribal Agency, which is in the neighbourhood of Kurram Agency (Riaz, 2006). A comprehensive report based on the analysis of meta data published in the literature until 2011 indicated different rates of zoonotic infections of brucellosis in human. For example, an incidence rate of 52.3 cases/1000000 person-years in Iraq, 18-70 cases

(depending on the region) per 1000,000 person-years in Egypt, while, 4-32 cases/100,000 person-years were reported in the western-central Europe (Dean et al., 2012). The increased rate of seropositivity in the females is most probably due to longer exposure to infected animals. Generally, domestic animals at home are handled by women during feeding and milking, thus increasing their chances of close physical contact with these animals. Taken together, our results indicate a high seroprevalence of brucellosis and a simultaneous seropositivity of high risk human raise concern for public health.

Conclusion

Kurram tribal Agency located in the North-west of Pakistan sharing its border with Afghanistan with people and animals moving freely between the boarders. Considering the endemic situation of brucellosis in Pakistan, the current reported seropositivity indicates a high level as reported in other parts of the country. However, transmission of brucella infection from livestock animals to human population and particularly those at high high risk associated human beings is quite alarming. Therefore, an immediate urgent action to control and eradicate the disease is required along with mass education campaign.

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Conflict of interests: Nothing to declare

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บทคัดย่อ

การติดเชื้อบรูเซลโลซิส ในคนและสัตว์เลี้ยงในเขตเฝ้าโครัน ปากีสถาน

บ่งบอกถึงการติดเชื้อข้ามในคนและสัตว์

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โรคบรูเซลโลซิสเป็นโรคประจำถิ่นในประเทศปากีสถาน อย่างไรก็ตามข้อมูลการเฝ้าระวังโรคบรูเซลโลซิสของประเทศยังขาดหายไป การศึกษาครั้งนี้มีวัตถุประสงค์เพื่อทราบความชุกทางซีรัมวิทยาในปศุสัตว์และคนที่มีความเสี่ยงติดโรค ในเขตเฝ้าโครัน ประเทศปากีสถาน โดยทดสอบตัวอย่างซีรัมสัตว์จำนวน 567 ตัวอย่าง (แบ่งเป็น โค 148 ตัวอย่าง, กระบือ 105 ตัวอย่าง, แกะ 154 ตัวอย่าง และ แพะ 160 ตัวอย่าง) และตัวอย่างซีรัมคนจำนวน 197 ตัวอย่าง (แบ่งเป็นกลุ่มที่มีความเสี่ยงต่ำจำนวน 83 ตัวอย่าง และกลุ่มที่มีความเสี่ยงสูงจำนวน 114 ตัวอย่าง) รวมทั้งศึกษาตัวอย่างซีรัมจากสัตว์เพศเมียที่มีประวัติคลอดใน 4 สัปดาห์ ทั้งที่มีหรือไม่มี การแท้งจำนวน 395 ตัวอย่าง โดยตัวอย่างทั้งหมดจะตรวจคัดกรองด้วยวิธี rose bengal plate test (RBT) และตรวจยืนยันด้วยวิธี indirect enzyme-linked immunosorbent assay (I-ELISA) ผลการศึกษาพบความชุกของโรคบรูเซลโลซิส ในโค 4.73% กระบือ 4.76% แกะ 1.95% และแพะ 3.13% และที่น่าสนใจพบความชุกของโรคบรูเซลโลซิส ในวัวแกะและแพะเพศเมียมากกว่าเพศผู้ ในขณะที่พบความชุกในกระบือเพศผู้มากกว่าเพศเมีย นอกจากนี้ยังพบความสัมพันธ์กันระหว่างการเกิดโรคบรูเซลโลซิส และการแท้งในสัตว์อย่างมีนัยสำคัญทางสถิติ และผลการศึกษาในคนพบว่า กลุ่มที่มีความเสี่ยงสูงมีความชุกของโรค 4.39% ส่วนกลุ่มที่มีความเสี่ยงต่ำมีความชุก 1.20% ที่น่าสนใจพบความชุกของโรคบรูเซลโลซิส ในคนเพศหญิงสูงกว่าเพศชาย ผลการศึกษานี้แสดงให้เห็นถึงความชุกในการติดเชื้อบรูเซลโลซิสสูงในคนและสัตว์เลี้ยง

คำสำคัญ: บรูเซลโลซิส ปศุสัตว์ ความชุกทางซีรัมวิทยา เขตโครัน ปากีสถาน

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