Seroprevalence and Molecular Diagnosis of Brucellosis Among Patients at Selected Hospitals of Abbottabad, Pakistan

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Abstract

Background: Brucellosis is a neglected disease of ruminants with zoonotic potential. It causes severe health problems in humans and economic loss. Only a limited number of studies have been conducted in Pakistan to determine the prevalence of human brucellosis and related risk factors. The objectives of the current cross-sectional study were to determine prevalence of *Brucella* infection in sera collected from patients at three hospitals of Abbottabad. Risk factors were investigated.

Methods: A total of 500 blood samples were collected. A questionnaire was filled in for each patient to obtain information on age, gender, living area, brucellosis associated symptoms, associated risk factors, pregnancy and abortion history. Serum agglutination test (SAT) was performed to detect antibodies against brucellae. A genus specific BCSP-31 gene RT-PCR was used to detect *Brucella* DNA in the sample. Statistical analysis was done to determine odd ratios, risk ratios, 95% confidence intervals and p values.

Results: A total of 13.6% (n=68) patients were found to be SAT positive. DNA was found in 11.4% (n=57) samples. The prevalence of brucellosis was reported to be higher in women (14.6%, n=44) than in man (12.1%, n=24). The age group of 25-50 years was found to be at higher risk for brucellosis (14.5%, n=50). “Animal contact” was reported as the main risk factor followed by “consumption of raw animal products”. About 9.9% pregnant women (n=13) were found brucellosis positive. Of these 23.8% (n=5) had an abortion history.

Conclusions: The present study reports a striking prevalence of brucellosis among patients including pregnant women at three hospitals of Abbottabad. These findings must foster strategies for controlling human brucellosis at household level, raising of awareness about brucellosis in hospital and family doctors and finally in setting up an eradication program in the dairy industry.

Background

Brucellosis is a disease caused by bacteria of species of the genus *Brucella* with a high zoonotic potential [1]. In developing countries, the disease is of great importance for public and veterinary health [2] by affecting both, human and animal health [3]. Endemic areas include the Mediterranean region, the Middle East, the Arab peninsula, Africa, Latin America and Asia [4]. Four species of *Brucella* (*B. abortus, B. melitensis, B. suis, B. canis*) are known to cause disease also in humans regularly. Other *Brucella* species i.e. *B. inopinata, B. cetaceae* and *B. microti* cause disease in animals but rarely in humans [5]. The number of new *Brucella* infections in humans exceeds 500,000 cases per year worldwide [4].

Bacteria of the genus *Brucella* are Gram negative coccobacilli without capsule and slow growing organisms [6]. They are aerobic and some are capnophilic. *Brucella* are facultative intracellular pathogens and have the ability to survive and propagate inside epithelial cells, macrophages, dendritic cells and placental trophoblasts [7]. Their main virulence factor and antigen which is important for survival in the host is liopolysaccharide (LPS) which is involved in the prevention of apoptosis of infected

cells [8]. Cytoplasmic antigens, structural proteins of the outer membrane such as OMPs and periplasmic proteins are antigens identified by the immune system of the host [9].

Brucellosis is a zoonotic disease. It is transmitted from animals to humans through ingestion of contaminated raw meat and unpasteurized dairy products like soft cheese and raw milk usually. It is also an occupational hazard for people with close contact to infected animals. They get infected via skin abrasion and cuts or inoculation. Inhalation of contaminated aerosols during bacterial culture has caused disease in laboratory staff. Brucellae can actively penetrate conjunctiva of the eyes. While transmission by person to person is rare [10]. Thus, brucellosis in humans mostly coincides with endemic infection of livestock [4].

Some *Brucella* species are highly infectious and are able to cause human brucellosis with an infection dose of less than 10 organisms [11]. The incubation period of brucellosis varies and the average incubation period is 2 to 3 weeks in man [12]. Brucellosis in humans is commonly known as "Malta fever" or "Undulant fever" and has been found to be one of the main causes of fever of unknown origin in endemic areas [13]. In humans, brucellosis causes undulant fever (39–40 °C), sweating, headache, fatigue, malaise, anorexia, weakness, arthralgia, back and abdominal pain. Symptoms may be present for some weeks or may get chronic or latent for months to years. In case of absence of proper treatment, severe complications may be seen [14]. The infection can affect any system or organ due to dissemination of bacteria via the blood [11]. Osteoarticular, hematologic, gastrointestinal, genitourinary, respiratory, cardiovascular and neurologic disorders can be provoked. Osteoarticular disorders are most common. The highest mortality is caused by endocarditis [9]. During the first trimester, spontaneous abortion and in utero death of the fetus in pregnant women have been documented [15]. In animals, the disease causes orchitis, infertility, abortion and reduced production of milk [3].

Due to the non-specific symptoms of the disease, brucellosis is misdiagnosed with other diseases such as typhoid fever, tuberculosis, malaria, leishmaniasis, malignancy and rheumatic fever [9]. Final diagnosis, therefore, must rely on laboratory techniques including bacterial isolation by culture, phenotypic or genotypic confirmation, and testing for *Brucella* antibodies by serological methods. Various serological methods are available but there is a lack of a perfect method. The specificities of serological tests are low with sensitivities ranging from 65–95% [16]. Nowadays, molecular techniques are used to detect DNA circulating in the blood stream to amend serology. These techniques have high specificities and are rapid [17].

Treatment of human brucellosis needs combination of antibiotics. Doxycycline with gentamycin or rifampicin is most commonly used for treatment [18]. Currently, doxycycline in combination with streptomycin is considered the best choice of therapy mainly for localized brucellosis and acute cases having less relapses and side effects [19].

There is no effective vaccine which has been approved to prevent brucellosis in humans. Therefore, control of human brucellosis depends on its prevention in animals to reduce exposure [20]. In endemic areas, food hygiene, i.e. pasteurizing dairy products is an important measure of safety. The infection risk

is also reduced through introduction of other biosafety and biosecurity measures such as personal hygiene, adopting safe practices of working or environment protection [21]. Water sources can be prevented from contamination through burying and proper handling of abortion material [22]. Vaccination in animals can reduce financial losses but is not an appropriate measure for eradication. In final steps of eradication, test and slaughter policy has to be applied [23].

According to OIE (Office for International des Épizooties, Paris), brucellosis is a neglected zoonotic disease which has a negative impact on human health and production of animals [19]. In Pakistan, multiple zoonotic diseases (i.e. toxoplasmosis, brucellosis etc.) are prevalent in the human population [24, 25]. Because brucellosis has a considerable impact on animal production and human health, the socioeconomic life situation of people of rural regions who mainly depend on cultivation of land and livestock rearing is substantially impaired [26]. Few studies have been conducted in Khyber Pakhtunkhwa province of Pakistan to investigate the prevalence of and risk factors for *Brucella* infection in patients of hospitals of Abbottabad.

Methods

Study area

The study was carried out at Ayub Medical Hospital, Jinnah Medical Hospital and DHQ of Abbottabad city. Abbottabad city is the capital of the district Abbottabad located in the Hazara region, province Khyber Pakhtunkhwa. It has a total area of about 1,967 km^2 or 759 square miles. According to the census of 2017, the total population is 1,332,912 and the density is 680 inhabitants per km^2 [27]. The total number of households of the district reporting livestock such as cattle, buffaloes, sheep, goats and camels etc. is 1,263,547 and the total number of reported animals of the KPK province is 5,967,886 according to data of 2006 [28]. Most of the rural population has to do livestock farming as there is little land available for agriculture in this district. Thus, a higher risk of acquiring brucellosis due to close contact to livestock can be supposed [29].

Collection of patients detail data by questionnaire

A questionnaire was filled in personally for each patient. Questions on age, gender, dwelling area, animal ownership or presence of animals in household, contact with animals, processing or handling raw animal products or meat, consumption of raw animal products, access of livestock to the household's source of drinking water, abortion in animals or contact with aborted animals, presence or previous history of symptoms such as fever, night sweats, head ache, arthralgia, generalized ache, nausea, anorexia and fatigue and presence of such symptoms or brucellosis in any other house-hold member had to be answered. Women were asked to report on previous pregnancies and abortion history.

Blood collection

500 blood samples from persons who visited the Outdoor Patient departments (OPD) of the hospitals and agreed to take part in this study were collected from April, 2019 to August, 2019. About 4 ml blood was collected aseptically from the brachial vein with disposable and sterile syringes. Blood was immediately injected and transferred into serum separating gel-tubes and tubes were labeled immediately. The serum was obtained by centrifugation at 6,000 rpm for five minutes. Each serum sample was divided into two parts for serum agglutination testing and DNA extraction to perform RT-PCR, respectively.

Serology

The *Brucella abortus* antigen of the Febrile Antigen Kit (Plasmatec, Lab21 Healthcare Ltd, Bridport, Dorset, United Kingdom) was used for serum agglutination slide test as per manufacturer instructions. Briefly, 80 µl, 40 µl, 20 µl, 10 µl and 5 µl of undiluted serum was added onto a row of 3 cm diameter circles of a reaction slide. Then a drop of the undiluted suspension of antigen was added to each serum sample by using the dropper provided with the kit. The content was mixed using a stirring stick. The slide was shaken gently for one minute and then observed for any agglutination. A test was positive when agglutination was observed at 1:160.

DNA extraction and quantification

DNA was extracted from serum samples by using WizPrep gDNA Mini Kit (Wizbiosolutions Inc. Jungwon-gu, Seongnam, South Korea) according to the instructions and protocol of the kit manufacturer. After extraction of DNA from serum samples, Nanodrop-1000 UV spectrophotometer (Nano-Drop technologies, Wilmington, DE) was used for DNA quantification. DNA quantification was performed by measuring absorbance at 260 nm and DNA purity was checked with the ratio of 260/280. A value of approximately 1.8 was considered to show pure DNA. The purified DNA samples were stored at -20 °C.

Real time-polymerase chain reaction

RT-PCR was done on a MJ Mini Bio-RAD Thermal cycler (Applied Biosystems, Foster City, California, USA). Genus specific primers and probes targeting the BCSP-31 gene were used according to [30]. The BCSP-31 gene codes for a 31KDa immunogenic protein of the membrane and is conserved among all *Brucella* species and biovars. The sequences of primers and fluorescent tagged probe are given in the Table 1.

Table 1
Sequences of Probe and Primers for genus specific *Brucella* RT-PCR

Target Gene	Probe and Primers	Sequences
BCSP-31 gene	Probe	5'-FAM-AAATCTTCCACCTTGCCCTTG CCATCA-BHQ1-3'
	Forward Primer	5'-GCTCGGTTGCCAATATCAATGC – 3'
	Reverse Primer	5'-GGGTAAAGCGTCGCCAGAAG – 3'

A total of 25 µl of reaction mixture was prepared for the amplification of each sample. The reaction mixture was prepared by adding 5 µl of 5x Amplicon qPCR master mix (Solis BioDyne, Teaduspargi, Tartu, Estonia), 0.8 µl forward primer (10 pmol/µl), 0.8 µl reverse primer (10 pmol/µl), 0.4 µl probe (5 pmol/µl), 3 µl extracted DNA sample and 15 µl of nuclease free water to a final volume of 25 µl.

The PCR conditions were: initial denaturation for 10 minutes at 95 °C, 44 cycles of 20 seconds at 95 °C for denaturation, 50 seconds at 60 °C for primer annealing and 50 seconds at 72 °C for DNA extension. The results were considered positive when the cutoff value was ≤ 40 cycles.

Statistical analysis

Data were statistically analyzed by using the online tools of Vassar Stats (Vassar College; Poughkeepsie, NY USA; http://vassarstats.net/). Collected data and results were categorized into groups. Version 2 software was used for analysis of logistic regression to determine odd ratio, risk ratio, 95% confidence interval and Chi-square test for p-value. Fisher exact test was used in case when the cross table had 5 or less counts. The data were considered to be statistically significant with a p-value ≤ 0.05.

Results

Out of 500 samples, 68 samples were found to be SAT positive. While 57 samples were confirmed positive by RT-PCR.

The associations of demographic factors with seropositivity for *Brucella* antibodies are given in Table 2. The study showed that the prevalence of brucellosis was higher in the age group 25–50 years (n = 50). The prevalence of brucellosis was 12.1% (n = 24) in males and 14.6% (n = 44) in females but this finding was not significant (p = 0.493). The prevalence of disease was reported to be 31.6% (n = 49) in participants of rural areas and 5.5% (n = 19) of urban area which was significant (p = < 0.0001).

Table 2
Association of demographic and epidemiological variables for seroprevalence of *Brucella* antibodies in the 500 tested patients from Abbottabad, Pakistan based on Chi-square analysis (2019)

Variables	Total Participants	Seropositive	Prevalence (%)	Chi-square	P-value
Age (Years)					
< 25	131	16	12.2	1.01	0.6035
25–50	345	50	14.5		
> 50	24	2	8.3		
Gender					
Male	199	24	12.1	0.47	0.493
Female	301	44	14.6		
Urbanicity					
Urban	345	19	5.5	59.83	0.0001
Rural	155	49	31.6		
Animals own/in house					
Yes	122	39	31.9	44.29	0.0001
No	378	29	7.7		
Animal Contact					
Yes	162	50	30.9	58.63	0.0001
No	338	18	5.3		
Processing/ Handling raw animal product/meat					
Yes	155	33	21.3	10.38	0.0013
No	345	35	10.1		
Consuming raw animal product					
Yes	117	28	23.9	12.75	0.0004
No	383	40	10.4		
Livestock access to source of drinking water					
Yes	73	25	34.2	28.99	0.0001
No	427	43	10.1		

Chi-square test was applied, *p-value less than 0.05 was considered statistically significant

Variables	Total Participants	Seropositive	Prevalence (%)	Chi-square	P-value
Contact with aborted animals					
Yes	33	13	39.4	17.72	0.0001
No	467	55	11.8		
Brucellosis related symptoms in any other family member					
Yes	81	21	25.9	11.28	0.0008
No	419	47	11.2		
Pregnancy status in Females					
Yes	131	13	9.9	3.46	0.0629
No	170	31	18.2		
Any Abortion History					
Yes	21	5	23.8	3.2	0.0544
No	110	8	7.2		
Chi-square test was applied, *p-value less than 0.05 was considered statistically significant					

Several risk factors that were related with the spread of brucellosis from animals to humans were determined (Tables 2 & 3). About 31.9% (n = 39) seropositive participants keep animals (cattle, goats, sheep etc.) at their homes which was found significant (p = 0.0001). The highest proportion of brucellosis (30.9%; n = 50) was observed among participants who had direct contact with livestock. This finding was significant (p = 0.0001). Processing or handling of raw animal products such as meat or milk etc. was also an important and significant factor (p = 0.0013) recorded for 33 (21.3%) patients. Consuming raw products of animals such as undercooked meat or unpasteurized milk was recorded for 28 patients (23.9%) and was found significant (p = 0.0004). 25 (34.2%) participants of the study reported that livestock had access to the source of their drinking water which was a significant finding (p = 0.0001). 13 (39.4%) participants had contact with material of aborted animals which was a significant finding (p = 0.0001). It was found that 21 (25.9%) of the patients had family members that had similar symptoms of brucellosis (p = 0.0008).

Table 3
Logistic Regression analysis to determine odd ratio, 95% Confidence interval and p-value between brucellosis positive cases

Variables	OR	95% CI	DF	P-Value
Gender	0.801	0.47–1.36	1	0.413
Area (Rural, Urban)	0.126	0.07–0.22	1	0.0001
Animals in house	5.65	3.30–9.67	1	0.0001
Animal contact	7.93	4.44–14.17	1	0.0001
Processing raw animal product	2.39	1.42–4.02	1	0.0007
Consuming raw animal product	2.69	1.57–4.61	1	0.0002
Livestock access to source of drinking water	4.65	2.61–8.28	1	0.0001
Contact with aborted animals	4.86	2.29–10.33	1	0.0001
Brucellosis related symptoms in any other family member	2.77	1.54–4.95	1	0.0004
Pregnant status in females	0.494	0.24–0.98	1	0.0428
Abortion history in pregnant females	3.98	1.15–13.70	1	0.0356

13 pregnant women were positive for brucellosis. The data analyzed were statistically non-significant between pregnant and non-pregnant women (p = 0.0629). 5 (23.8%) SAT positive pregnant women had also an abortion history which was found also significant (p = 0.0544).

The most common clinical signs observed in positive patients were fever 94.1% (n = 64), arthralgia 55.8% (n = 38), generalized ache 55.1% (n = 34), anorexia 47% (n = 32), head ache 32.3% (n = 22), fatigue 32.3% (n = 22), nausea 26.4% (n = 18) and the least common clinical sign observed was night sweat 25% (n = 17). The clinical symptoms observed in study participants are shown in Table 4.

Table 4
Clinical signs and symptoms of brucellosis in seropositive patients

Clinical Presentations	SAT Positive (n = 68)	Prevalence (%)
Fever	64	94.1
Night Sweats	17	25.0
Headache	22	32.3
Arthralgia	38	55.8
Generalized ache	34	55.1
Nausea	18	26.4
Anorexia	32	47.0
Fatigue	22	32.3

Discussion

Brucellosis is a zoonotic disease of worldwide distribution. It negatively impacts human health, and animal production and economy by significant loss [12]. Brucellosis may become chronic causing severe osteoarticular, cardiovascular, neurological and genitourinary complications including epididymo-orchitis and abortion in pregnant women if left untreated [31, 32]. Main reasons for human disease are animal brucellosis in bovines and small ruminants, several risky behaviors such as consumption of unpasteurized and contaminated dairy products and not wearing protective clothing when handling potentially infectious animals and their products [33].

In slaughterhouse workers, villagers and farm workers of rural Pakistan, who are considered to be at special risk to get infected with brucellosis, a seroprevalence of 14% was found [34]. These authors found a prevalence of 6.9% in a group at risk from the Potohar Plateau in 2013 [26]. In comparable settings in India [35] and in Bangladesh [36] prevalence's were reported to be 7.32% and 4.4%, respectively. It is also well known that a significant number of brucellosis cases can be found in patients with fever of unknown origin in endemic countries. Involving 7567 patients with suspect, yearly prevalence's from 10.4 to 15.7% were found at a Saudi hospital between 2014 and 2018 (37). At Ayub Teaching Hospital, Abbottabad 70 patients from different districts presenting with nonspecific symptoms (fever, body aches, myalgias, arthralgia, headache, backache, malaise and insomnia) were recently screened with SAT (cutoff titer > 80) for *Brucella* antibodies and amazingly 49 were found positive (38). In contrast to that extraordinary high prevalence these authors documented a SAT seroprevalence of 13.6% (n = 68) in persons seeking advice at three different outdoor hospitals from Abbottabad. This prevalence shows the high burden of disease in the rural population although it could not be figured out how many admissions were brucellosis related indeed. The strongly diverging results of the two preliminary studies from Abbottabad show the need for future research to provide sustainable data for public health use. It has to be stressed that data from

different studies cannot be compared without caution as various not standardized or harmonized tests are still used and those tests are not used on a routine basis at the labs either. Hence, these data show that physicians at hospitals in endemic areas should be aware of brucellosis in their day to day work.

The prevalence of brucellosis was highest in the age group 25–50 years. This finding can be explained by the fact that participants of this middle-aged group were mainly veterinarians, butchers and milking personnel who were in close contact to animals. However, seropositive cases of brucellosis were reported in participants of all age groups. As already stressed different studies cannot not be compared due to the variety of techniques used. So, the highest prevalence was found in the age group of 20–30 years (26.92%) while low prevalence was recorded in older age > 40 years (7.80%) for study participants from the Punjab, Pakistan [39 Ali 2018]. An Indian study found persons of age group 26–35 years affected most (10.8%) [40]. A study conducted in Southern Saudi Arabia among febrile patients determined the highest seroprevalence in patients 21 and 40 years of age (35.8–45.3%), while low prevalence was recorded in young children and older people (3 and 15%), respectively [37]. More positive cases were found in the age group of 41–80 years in Bangladesh which contrasts with our study [36]. Hence, an interpretation of the data points to the fact, that in rural populations the presence of antibodies comes along with contact to brucellae or their LPS at work and so it is very likely to find more positives aged from 15 to 55 years. Neither the fact that anti-*Brucella* antibodies are detected in study participants nor that patients at hospitals are involved allow the final statement that an active infection caused those due to the shortcomings of brucellosis serology. The interpretation of these data has to be done in the light of the epidemiological context. Trends, however, are obvious and can be used to guide countermeasures.

Brucella infection was found more often in female than in male patients. Similarly, a higher prevalence of brucellosis in female patients (37%) was also recorded from Peshawar [41]. The explanation is that animal husbandry is done mainly by women in Pakistan. Therefore, they are in direct contact to animals during their daily activities and also help during parturition without using precautionary measures. In contrast, all seropositive patients were reported to be man in a study conducted among persons of Ludhiana, India where only few women were involved in activities that exposed them to animals and other potential risk-factors [40]. Similarly, a study conducted in high-risk group persons from Bangladesh also found a higher prevalence in man (5.6%) than in women (0.8%). The main reason for this finding was, that mainly butchers, milkers, livestock farmers and veterinary practitioners were tested. These occupations are traditionally in the hands of men there and expose them to a high risk of infection [36]. This is also true for Egyptian setting where more man is involved in management of livestock [42].

This study found a higher prevalence in persons from the rural area demonstrating a higher risk of acquiring brucellosis than for people of the urban area. This finding is similar to that of a study conducted in Peshawar among hospital patients [41]. Persons from rural areas are often involved in birthing and herding of livestock and they are more dependent on livestock production putting them at the risk of infection [39]. A cross sectional study was conducted on rural population of the Punjab in India and seropositivity was also linked to a history assisting with abortions and calving [43].

Nicoletti stated that each case of brucellosis in humans is related to an animal source and its presence in animals causes a major risk of *Brucella* infection for humans [44]. Thus, animal contacts, processing or handling raw animal products or meat and consumption of raw foods are the main risk factors to be considered [45, 46]. Additionally, the risk factor “access of livestock to source of drinking water” was considered to take into account epidemiological circumstances. Indeed, this study showed the highest seroprevalence in the group of persons who had direct contact with livestock (30.9%) or raised livestock at home (31.9%). As expected, processing or handling raw animal foods proved to be an important risk factor (21.3%). 28 patients recorded consumption of undercooked meat or unpasteurized milk. 13 (39.4%) participants had direct contact with materials of aborted animals. Aborted foeti are usually left for decomposition by scavengers instead of proper disposing. This procedure increases the infection risk because large numbers of organisms are excreted with the uterine fluid, placenta and fetus at the calving/lambing time [47].

Brucellosis in pregnant women bares the risk of abortion and may also cause repeatedly abortions after becoming chronic. Thus, participants of this study were asked about the course of previous pregnancies. Indeed, 23.8% (n = 5) seropositive women reported on abortion. A recent study in Pakistan involving 429 pregnant women mostly from rural areas reported 5.8% seroprevalence and 14.6% of these had abortion history [24]. Due to the low number of cases in both studies, we only can recommend further studies to evaluate these findings Brucellosis can also pose a serious risk to newborns. Mortality in newborns was reported as well as transmission of brucellosis to a neonate via the congenital route or via breastmilk [48–52]. Future studies in Pakistan should also consider this neglected aspect of brucellosis in childhood. 47 reference deleted

The most common clinical sign observed among SAT seropositive patients was fever i.e. 94.1% (n = 64), followed by arthralgia with 55.8% (n = 38). Similarly, most often fever (77.71%) and arthritis (83.43%) were reported in 175 brucellosis positive hospital patients in Bikaner, India [53]. 30.8% (n = 21) participants of this study reported persons with similar symptoms of brucellosis in their households. Transmission from person to person is rare, so these household members were most probably infected by the same animals or foods. Brucellosis in endemic countries is a family problem. Physicians should be aware of that fact and include all family members in their investigations. As brucellosis can be attracted again and again from the same source it is of imminent importance to identify and eliminate this source as well. Patients must be made aware of the epidemiology of brucellosis and local veterinary officers need to be involved finally.

RT-PCR was performed for confirmation of brucellosis in patient samples. RT-PCR is a rapid, reliable, highly sensitive and specific method for molecular diagnosis. Genus specific primers and probes targeting the conserved BCSP-31 gene were used. 11.4% samples were RT-PCR positive. The study showed that RT-PCR is a rapid method to confirm brucellosis within 2–3 hours as compared to conventional methods which take several days to weeks and also poses a high risk of infection to laboratory personnel. Cases of brucellosis might have been lost by serological tests because they were in early stage of infection and antibodies were absent still.

Conclusions

The present study reported the prevalence of brucellosis among patients including pregnant women from hospitals of Abbottabad, Pakistan. The study showed that the population of Abbottabad is at higher risk of acquiring brucellosis because most people although living in urban areas have close contact with animals and consume raw products of animal's e.g. unpasteurized milk. The results of this study can be used to develop strategies for controlling human brucellosis in rural settings of Pakistan, to raise awareness about brucellosis in livestock professionals, consumers and physicians and to develop control programs by the authority in charge.

Abbreviations

SAT: Serum agglutination test; BCSP-31: Brucella cell-surface protein 31; RT-PCR: Real-time polymerase chain reaction; DNA: deoxyribonucleic acid; OIE: Office for International des Épizooties; DHQ: District Headquarters; KPK: Khyber Pakhtun Khwa; OPD: Outdoor Patient departments.

Declarations

Ethical Approval and Consent to participate

The study was approved by the ethical committee of the University of Haripur, Haripur, Pakistan (Approval Number: F. No (01) ORIC-UOH//2020/). The oral and written consent was taken from each patient before sample collection.

Consent for publication

Not applicable.

Availability of supporting data

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

LH, SA, MAS, FM, HEA and HN conceptualized the study and did the manuscript write-up. LH, ST, US, AAS, SAA and GM analyzed the data. LH, SA, MAS, US and AUK wrote the article. All authors read and approved the final manuscript.

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