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Short Communication

Prevalence of Brucellosis in Human Population of District Swat, Pakistan

Hussain Ahmad[1], Inamullah[1], Ijaz Ali[2], Tauseef Ahmad[3], Muhammad Tufail[4], Kabir Ahmad[5] and Bibi Nazia Murtaza[3,6]*

[1]*Department of Genetics, Hazara University, Khyber Pakhtunkhwa, Pakistan*
[2]*Institute of Biotechnology and Genetic Engineering, Khyber Pakhtunkhwa Agricultural University, Peshawar, Pakistan*
[3]*Department of Microbiology, Hazara University, Mansehra, Khyber Pakhtunkhwa, Pakistan*
[4]*Department of Health, Khyber Pakhtunkhwa, Pakistan*
[5]*Department of Zoology, Hazara University, Mansehra, Khyber Pakhtunkhwa*
[6]*Department of Zoology, Kinnaird College for Women, Lahore, Pakistan*

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Authors' Contributions
HA, I and BNM conceived and designed the study. HA, IA, TA, MT and KA collected samples from the hospitals and performed the experiments. MA, IA and BNM analyzed the data. HA and BNM wrote the article. HA and BNM supervised the work.

Key words
Brucellosis, Prevalence, Standard Plate Agglutination test, Serum tube agglutination test.

ABSTRACT

Brucellosis is a zoonotic disease affecting several animal species and human beings. It is caused by *Brucella abortus* in animals and *Brucella melitensis* in human. The current study aimed to find out the prevalence of *Brucella* and active brucellosis in the human population of district Swat. Total 300 individuals (both female and male of all ages, meeting our inclusion/exclusion criteria) with informed consent were included in the study. Blood samples were collected and brucellosis was detected by Standard Plate Agglutination Test (SPAT) and Serum Tube Agglutination Test (STAT) and later on confirmed by PCR. Disease was detected in 3.66% (11/300) individuals by SPAT and 2% (6/300) by STAT and confirmed in 2.66 % (8/300) by PCR. The prevalence of disease in females was found to be higher as compared to males (in current studied group). The age group 31-40 years, was found to be more susceptible for this infection.

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Brucellosis caused by bacterial genus *Brucella,* is one of the common zoonotic infections. Pathogenic *Brucella* species in humans are *B. melitensis* (animal reservoirs is sheep and goats), *B. abortus* (animal reservoirs is in cattle), and *B. suis* (animal reservoirs is in swine). *B. abortus* and *B. melitensis* species are genetically very similar to each other. In humans, it causes undulant fever and male sterility and in animals, *B. abortus* causes abortion. Aerobic gram-negative *Brucella* has the ability to invade both phagocytic and nonphagocytic cells. It has the ability to survive in the intracellular environment by finding ways to avoid the immune system (Lecaroz *et al*., 2006). Transmission of infection can be through direct contact with infected animals, inhalation of airborne agents or usage of unpasteurized milk or cheese (Pappas *et al*., 2006). The disease causes various symptoms including weakness, periodic fever, muscle aches, malaise, weight loss and fatigue (WHO, 2006). Generally brucellosis is diagnosed serologically by detection of agglutinating antibodies (IgM, IgG, and IgA classes) (Godfroid *et al*., 2005). The most frequently used serological tests are Rose Bengal test (RBT), the tube agglutination test (TAT) and the complement fixation test (CFT) (Manat *et al*., 2016). Control of the disease depends on the system of animal management in the region and available epidemiological information about the disease. For effective control of this disease in Pakistan, Brucellosis control programs should

* Corresponding author: nazia.murtaza@gmail.com
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be initiated (Ali *et al*., 2013). Current study was designed to estimate the prevalence of disease in human population of District Swat, Pakistan.

Materials and methods

Blood samples were collected from Saidu Group of Teaching Hospitals, Swat and Tajwar Sultana Hospital Swat, Khyber Pakhtunkhwa (KP), Pakistan (Table I). The study was conducted during March 2012 to Jan. 2013.

Patients of both genders of all age groups, visiting to seek the medical help with the symptoms suspected for brucellosis (periodic fever, muscle aches *etc.*), selected by the clinicians, were included in the study. Patients having tuberculosis or with the history of tuberculosis were not included in the study.

To collect the necessary information about the participants**,** a questionnaire was designed. An informed consent was signed by all the participants and they were ensured about the confidentiality of results and privacy of the hospital record.

From each participant, 5 ml blood was drawn out and transported to Institute of Biotechnology and Genetic Engineering, Peshawar. Each sample was centrifuged for 5 min at 5514 x g and separated serum was stored at -20°C for further analysis.

Two serological tests, SPAT (Co., Inc. Morganville, N.J. 07751) and STAT (Co., Inc. Morganville, N.J. 07751) were used for the diagnosis of brucellosis.

Samples showing positive results with SPAT and STAT were further processed for molecular analysis by PCR. Amplification of small fragment of *Brucella* genome was done by using *Brucella* PCR kit (Shangai ZJ Bio-tech Co. Ltd China) according to the recommended procedure established by manufactures with little modifications.

DNA was extracted out from 50µl blood serum. Master mix of volume 34.4µl was prepared by adding 34µl reaction mix and 0.4µl enzyme (provided in the kit). To 35µl of master mix, 5µl DNA sample was added. Initial denaturation was carried out at 94°C for 2 min followed by 35 cycles with denaturation at 93°C for 15 seconds, annealing at 55°C for 30 seconds and extension at 72°C for 30 seconds. Final extension was carried out at 72°C for 10 min.

All categorical variables such as age and gender were compared and analyzed by chi-square test. Results were considered significant when *p* value was less than 0.05.

Results

Total 300 serum samples were examined for the prevalence of brucellosis (Table I). Brucellosis was detected in 3.66 % (11/300) samples by SPAT and 2% (6/300) by STAT. The presence of diseases was confirmed by PCR in 2.66 % (8/300) of total studied population. The registered patients were categorized into two age groups. Individuals <30 years of age were placed in group 1 and ≥31years of age were in group 2. In group 1, 0.62 % (1/160) samples showed positive results by SPAT while STAT failed to detect brucellosis in this group. In group 2, 7% (10/140) samples showed positive result by SPAT while 4 % (6/140) of samples showed positive result by STAT. It was observed that 3% of male patients were brucellosis positive by SPAT and 2% by STAT (Table II). When area wise distribution of disease was assessed, out of total 8 confirmed brucellosis patients, 6 were from Khuwza khelaa (K. khela), 1 from Babuzai and 1 from Kalam (Table III).

Table I.- Sample collection from different regions of District Swat.

Name of Tehsil	Male	Female	Total
Babuzai	10	20	30
Charbagh	15	25	40
Khuwza khelaa	25	35	60
Mata	15	39	54
Bahrain	8	16	24
Kalam	4	12	16
Kabal	15	35	50
Barikot	8	18	26
Total	100	200	300

Table II.- Prevalence of brucellosis in association with different demographic characteristics.

Parameters	Total out of 300 individuals	Cases positive by SPAT (n= 11)	Cases positive by STAT (n=6)	P value
Age				
G1 <30	160	1	0	0.44
G 2 ≥31	140	10	6	
Gender				
(Men)	100	3	2	0.81
(Women)	200	8	4	
Education				
Illiterate	250	10	5	0.04
Literate	50	1	1	

Discussion

Scientists and clinicians are still facing several challenges in understanding the pathogenic mechanisms

Table III.- Analysis of brucellosis in Swat.

Code No	Age	Gender	Area	SPAT	STAT	PCR
B22	30	M	Babuzai	+	-	+
B37	40	M	K. Khelaa	+	+	+
B101	35	F	Kabal	+	-	-
B105	37	F	Matta	+	-	-
B138	35	F	Matta	+	+	+
B144	40	F	K. Khelaa	+	+	+
B153	40	F	K. Khelaa	+	+	+
B175	32	F	K. Khelaa	+	-	-
B190	45	F	K. Khelaa	+	+	+
B211	40	F	Kalam	+	+	+
B213	50	M	K. Khelaa	+	-	+

of human brucellosis and the identification of markers for disease severity and progression (Franco *et al*., 2007). Untreated human brucellosis can be fatal or ends with serious health complications. *I*nfection in brucellosis is primarily controlled through cell-mediated immunity system of the body. To control reinfection, IgM levels rise, followed by IgG titers (Lecaroz *et al*., 2005; Bouza *et al*., 2005).

In present study, brucellosis was detected in 3.66% (11/300) samples by SPAT and 2% (6/300) by STAT. The presence of diseases was confirmed by PCR in 2.66% (8/300) of total studied population. The highest active human brucellosis was observed in the individuals between 31-40 years of age. The disease is more common in female as compared to male. Generally it is found to be more common in males than in females. Fallatah *et al*. (2005) found male-to-female ratio of 1.7:1 individuals aged 13-40 years in Saudi Arabia. High seroprevalence of brucellosis was observed in humans aged above 30 years by Nahar and Ahmed (2009). Shafee *et al*. (2012) studied the prevalence of the disease in cattle and buffaloes by RBPT (3%) and through i-ELISA (3.20%) in Quetta Balochistan. Hussain *et al*. (2008) observed 4%, 3% and 3.33% seroprevalence of brucellosis in goats by Rose Bengal Plate Test (RBPT), Serum Agglutination Test (SAT) and Milk Ring Test (MRT), respectively.

Lack of education and proper awareness about health and diet is the major reason for infections in the area. When presence of infection was assessed with education level, 83% of total patients (seeking medical help with related symptoms) found to be illiterate. As the presence of brucellosis in animals has direct effect on human brucellosis so vaccination in animals should be carried out to control the disease. People living in rural areas are consistently exposed to infected animal excreta and secretions during raising, milking and slaughtering of domestic animals (Smith and Kadri, 2005). There are different diagnostic tests being used in Pakistan. We used Standard Plate Agglutination Test (SPAT) and Serum Tube Agglutination Test (STAT) for initial diagnosis and confirmation was done by PCR. The variation in results highlights the need to establish an accurate reference diagnostic method to confirm the actual presence of brucellosis to avoid side effects of prolonged antimicrobial therapy. All the brucellosis patients should be tested every 3 to 6 month interval. Recently established molecular techniques should be used for accurate diagnosis (Azizpour *et al*., 2013; Cha *et al*., 2012; Christopher *et al*., 2010). Also we need to develop a coherent and long-term strategy for to increase the awareness about the transmission of disease.

Conclusions

The study showed that prevalence of the disease is more in female as compared to male. It can be inferred that brucellosis continues to be a public health concern in the rural area of Swat district such as Khuwzakhelaa and need a good applicable strategy to control and eradicate the disease from the area.

Statement of conflict of interest

The authors declare no conflict of interest regarding this paper.

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