Research Article

Evaluation of Brucellosis as a Public Health Hazard Under the Biorisk Management Perspective

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Abstract | Brucellosis is an emerging animal's as well as human health issue and holds an important position under the Biorisk Management framework and One Health perspective. A total of 410 and 202 blood samples were collected from cattle and humans respectively. Total 40 samples of subsistence farms and 30 nomadic herds were analysed. Forty eight farm workers, 40 farm owner 36 abattoirs workers, 15 veterinary assistants, 15 veterinarian and 40 butchers were also screen out. Overall sero-prevalence in cattle was 15.36% on SPAT, 14.39% by i-ELISA and 14.14% through PCR. Higher prevalence was recorded in abattoir shops (31.01%) followed by private farms (11%) and animal selling points (9.25%). Significant difference was recorded in different breeds i.e. achai, local non-descript and crossbred (non-descript x jersey) cattle. Higher prevalence in female cattle (15.14%) was recorded as compared to male (14%). Similarly significant difference was recorded among age group i.e. adult were more susceptible as compare to young ones. In subsistence farms level prevalence in intensive, extensive farming and nomads was 74.07%, 57% and 83.3% respectively. Breeding through natural service was in greater risk (94%) as compared to artificial insemination (78%). In humans overall sero-prevalence was recorded 12.12%, 09.59% and 08.59% by SPAT, i-ELISA and PCR respectively. Highest incidence was found in farm workers (14.5%) followed by butchers (10.5%), veterinary assistant (10%) and abattoir workers (08.33%). High incidence was observed in butchers 20.58%, followed by veterinary assistants 14.28%, farm workers 12.28%, abattoir workers 11.53%, farm owners 10.41% and no positive cases were found in veterinarians through PCR.

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Introduction

Bovine brucellosis is an infectious and highly contagious disease which mainly affects reproductive organs (John et al., 2002). Brucella abortus is the prime source of disease whereas brucella militensis is also responsible for infection (OIE, 2009). Clinically brucellosis is characterized by abortion, metritis, orchitis and epididymitis (Radostits et al., 2007). Brucellosis is also classified as one of the mistreated zoonoses with a serious public health importance worldwide (OIE, 2008; WHO, 2007). Developed countries have almost freed themselves from brucellosis through eradication campaigns (Makita et al., 2008). But it is still prevalent in Mediterranean basin, Middle East, Western and Central Asia, Latin America, Africa and India (Maurin and Maurin, 2005). Different types of risk factors are involved which influences the prevalence of brucellosis, some of these are, age, herd size and composition, hygienic status of the farm, rate of contact between infected animals, farm biosecurity, and climate (John et al, 2002; Radostits et al., 2007). The present study was design to investigate the sero-prevalence of bovine brucellosis in cattle and human asso-

ciated with livestock in District Swat.

The prevalence of brucellosis in Pakistan has been reported from 3.25 to 24.96% (Naeem et al., 1990). Compared to small dairy herds holdings large dairy herds possess more incidence ratio of B. abortus (Sheikh et al., 1967; Ahmad and Munir, 1995). *B. abortus* infects cattle and buffaloes much higher as compared to other livestock various government and private livestock farms. Compared to government farms Private livestock farms, showed higher percentage of seropositive cattle and buffaloes (Nasir et al., 2004). In Quetta Baluchistan the prevalence of *B. abortus* (8.5%) is higher than previously reported (3.97%) positive cases in cattle and buffaloes. As compared to buffaloes the prevalence is higher in cattle (Shafee et al., 2011). According to Hamidullah et al. (2009), In Kohat 17.58% and 32.5% sero-prevalence of brucellosis was recorded at various government and private farms in cattle and sheep/goat.

To manage brucellosis at local or national level, it is essential to diagnose it urgently and accurately. Various conventional and advance molecular techniques are in practice for diagnosis of brucellosis. No single serological test is appropriate in all epidemiological circumstances; every one of them has a number of restrictions predominantly for screening individual animals. All aspects should be under deliberations that have impact on the test results and method. The most appropriate screening tests are the Rose Bengal test (RBT) and the buffered plate agglutination test (BPAT), Enzyme Linked Immunosorbent assay (ELISA) and the fluorescence polarization assay (FPA). Some ELISAs and FPA have similar or better diagnostic performance as compared to complement fixation test (CFT) because they are simple, easy to perform, sensitive and preferred to use (OIE, 2009). All these conventional tests have some limitations, as brucellosis is caused by various species of brucella, sometime the different species shares common epitopes and does not give accurate diagnosis.

Materials and Methods

Present study was conducted in District Swat, Khyber Pakhtunkhwa. A total 384 samples from cattle and 173 for humans calculated through $N= (1.96)^2 \times PQ/D^2$ (Thrusfield, 1995). In this study, 410 and 202 blood samples were collected from cattle and humans. Forty subsistence farms and nomads coming around were screened randomly throughout District Swat. Farm workers, AI technician and butchers were also screened for *Brucellosis*. Three different farm sizes were categorized as: Small size (<5 cattle); medium size (5-10 cattle) and large size (>10 cattle). The collected samples were analyzed at Department of Animal Pathology and Institute of Biotechnology and Genetic Engineering (IBGE), The university of Agriculture Peshawar, Pakistan, during summer, 2013. Initially all the serum samples were screened for *Brucella Abortus* Ag through Serum Plate Agglutination Test (SPAT) and Indirect Enzyme Linked Immunossorbant Assay (i-ELISA) at Institute of Biotechnology and Genetic Engineering (IBGE). All positive samples for anti *Brucella abortus* were additional used for DNA extraction and qualitative PCR.

Serological Tests

Serum Plate Agglutination Test (SPAT) and Indirect Enzyme Linked Immunossorbant Assay (i-ELISA) were used. Initially all serum samples were screened on SPAT. After screening, all serum samples were confirmed through i-ELISA for *Brucella abortus* antibodies at Disease Investigation Laboratory in Livestock and Dairy Development Department Peshawar. The samples were also subjected to PCR for confirmation and identification of species of brucella. ELISA positive serum samples were subjected to PCR DNA thermal cycler (Biorad®).

Detection of PCR Products

Set of oligonucleotide primers, BA-f & BA-r for *Brucella Abortus* was used to amplify 285 bp of region.

5`-GGATCCCATCTCGACCACGAGAAAA-3`

and

3`- CTTTCAATCAGTGAGTAACTGATGA-5`

PCR amplified products was then run on 1.5% agarose gel along with 100 bp ladders (Gene Ruler, Fermentas) as a DNA marker.

Statistical Analysis

Risk factors and relationship of bovine brucellosis with various parameters both in cattle and human was descriptively analyzed through cross tabulation by chi-square test, using SPSS-16.

Results

Sero-prevalence of *Bovine brucellosis*

Sero-prevalence of brucellosis in individual cattle

was recorded 15.36%, 14.39% and 14.14% according to SPAT, i-ELISA and PCR respectively. The SPAT positive samples were screened by i-ELISA and found that out of 63 samples 59 (14.39%) were recorded positive. These 59 samples were further screened through PCR, showed 58 (14.14%) samples positive (Table 1). Prevalence of bovine brucellosis was recorded higher in animal slaughtering or abattoir shops (31.01%) followed by private farms (11%) and animal selling points (9.25%) according PCR. The prevalence rate among the mentioned three spots was highly significant (P=0.043) as shown in Table 2.

Table 1: *Sero-prevalence of positive samples of brucellosis investigated through various diagnostic tests in District Swat (n=410)*

Test type	SPAT	i-ELISA	PCR
No of Positive samples	63	59	58
Prevalence	15.36%	14.39%	14.14%

Table 2: *Bovine brucellosis prevalence in private farms, animal slaughtering and cutting units and animal selling points*

Sampling spot	Total Sample	PCR Positive	Prevalence(%)	P-value
Private farms	200	22	11.25	
Abattoir shop	147	22	31.07	0.043
Selling point	69	14	09.52	

Table 3: *Association of animal physiological status with prevalence of Bovine brucellosis*

Factors		Total Sample	PCR Positive	Prevalence(%)	P-value
Breed	Achai	181	17	09.39	0.04
	Cross	105	23	21.90	
	Undescript	124	18	14.51	
Sex	Male	86	12	14	0.06
	Female	324	48	15.14	
Age	<3 years	111	12	10.81	0.01
	3-5 years	137	18	13.13	
	>5 years	162	28	17.28	

Association of Animal Physiological Status with Sero-prevalence of *Bovine brucellosis*

Collected serum samples represent achai, local non-descript and crossbred (non-descript x jersey) cattle. Among these breeds, in Acahi cattle 17 positive cases out of 181 were reported with 09.39% prevalence. Eighteen positive cases out of 124 were observed in local non-descript cattle with 14.51% prevalence. Highest prevalence of bovine brucellosis was observed in crossbred cattle with 23 positive cases out of 105 cattle with 21.90% prevalence. Female cattle were more prone (15.14%) to bovine brucellosis as compared to male cattle (14%) as shown inTable 3. Similarly, cattle of age more than 5 years were more susceptible to bovine brucellosis as compare to lowered age cattle with 17.28% of sero-prevalence.

Farm Based Factors of *Bovine brucellosis*

Bovine brucellosis was more prevalent (83.3%) in cattle reared under nomadic system followed by intensive (74.07%) and extensive (57%) production system (Table 4).

Table 4: *Farm based factors of Bovine brucellosis*

Factors		Total Farm	PCR Positive	Prevalence(%)	P-value
Farming system	Intensive	27	20	74.07	0.059
	Extensive	23	13	57	
	Nomads	30	25	83.3	
Herd Size	<5 cattle	18	6	33.33	0.045
	05-10 cattle	28	16	57.14	
	>10 cattle	30	24	80.00	
Breeding Practice	AI	32	25	78.00	0.018
	Natural	35	33	94.20	

Co-relation among *Bovine brucellosis* and different Age and Duration of Animal Handlers

Among the occupational groups, farm workers and butchers have high infection rate (14.50%) and (10.5%), followed by veterinary assistants 10.0%, abattoir workers 8.33%, farm workers 13.88%, and no prevalence rate was recorded among veterinarians. Age wise, highest incidence rate 12.3% was found in human having age more than 40 years followed by age groups 25-40 years having 9.47% prevalence rate. The lowest prevalence 5.0% was found in humans having age less than 25 years. The prevalence rate 10.2% was found in human having job duration more than 20 years, followed by 9.80% and 7.16% in 10-20 years and <10 years job duration category, respectively as shown in Table 5.

Association of *Bovine brucellosis* with various Variables in Humans

Highest but non-significant association with bovine brucellosis was found by raw milk consumption 22.80%, followed by animal parturition 21.76%, animal slaughtering 12.50%, artificial insemination

11.42% and milking 11.25%. The result shows that highest but non-significant association with bovine brucellosis was found by raw milk consumption 22.80%, followed by animal parturition 21.76%, animal slaughtering 12.50%, artificial insemination 11.42% and milking 11.25% as shown in Table 6.

Specificity and Sensitivity of the PCR Assay

Specificity of PCR assay for *Brucella abortus* was determined by the ability of the *Brucella abortus* primers to specifically amplify *Brucella abortus* DNA in a PCR reaction. DNA from various viral and bacterial strains (Adeno virus, chicken anemia virus, avian pneumonia virus, *Mycoplasma gallisepticum* and *Mycoplasma synoviae*) were used in the PCR reaction along with *Brucella Abortus* primers. In none of the reactions with other viral and bacterial types, even non-specific amplification was detected while only the *Brucella abortus* genome was amplified. It reflected that these *Brucella abortus* primers are 100% specific for the amplification of *Brucella abortus* genome. Sensitivity of the assays was determined by serially diluting the *Brucella abortus* DNA. Initially, we determined the started the PCR reaction for *Brucella abortus* with 500 pg of DNA per reaction gives no amplification. Lowering the DNA concentration had a significant effect on PCR amplification. *Brucella abortus* DNA in the range of 10-100 pg could be detected easily as shown in Figure 1.

Table 5: *Prevalence risk of Bovine brucellosis to diverse group of animal handlers*

Factors		Total Sample	PCR Posi	Prevalence(%)	P-value
Occupational groups	Butchers	40	4	10.5	0.462
	Farm workers	48	07	14.5	
	Farm owner	40	1	02.5	
	Vet assistants	19	2	10.0	
	Abattoir	36	3	8.33	
	Veterinarian	15	0	0.00	
Age groups	<25 years	63	3	5.00	0.012
	25-40 years	95	09	9.47	
	>40 years	40	05	12.3	
Job duration	<10 years	98	7	7.16	0.001
	10-20 years	51	5	9.80	
	>20 years	49	5	10.2	

Vet: *veterinary*

Table 6: *Association of Bovine brucellosis with various variables in human*

Variables	Total Sample	PCR Positive	% age	P-value
Animals parturition	36	5	21.76	0.1
Animals slaughtering	28	3	12.50	
Milking	20	2	11.25	
Artificial Insemination	17	2	11.42	
Raw milk consumption	87	3	22.80	

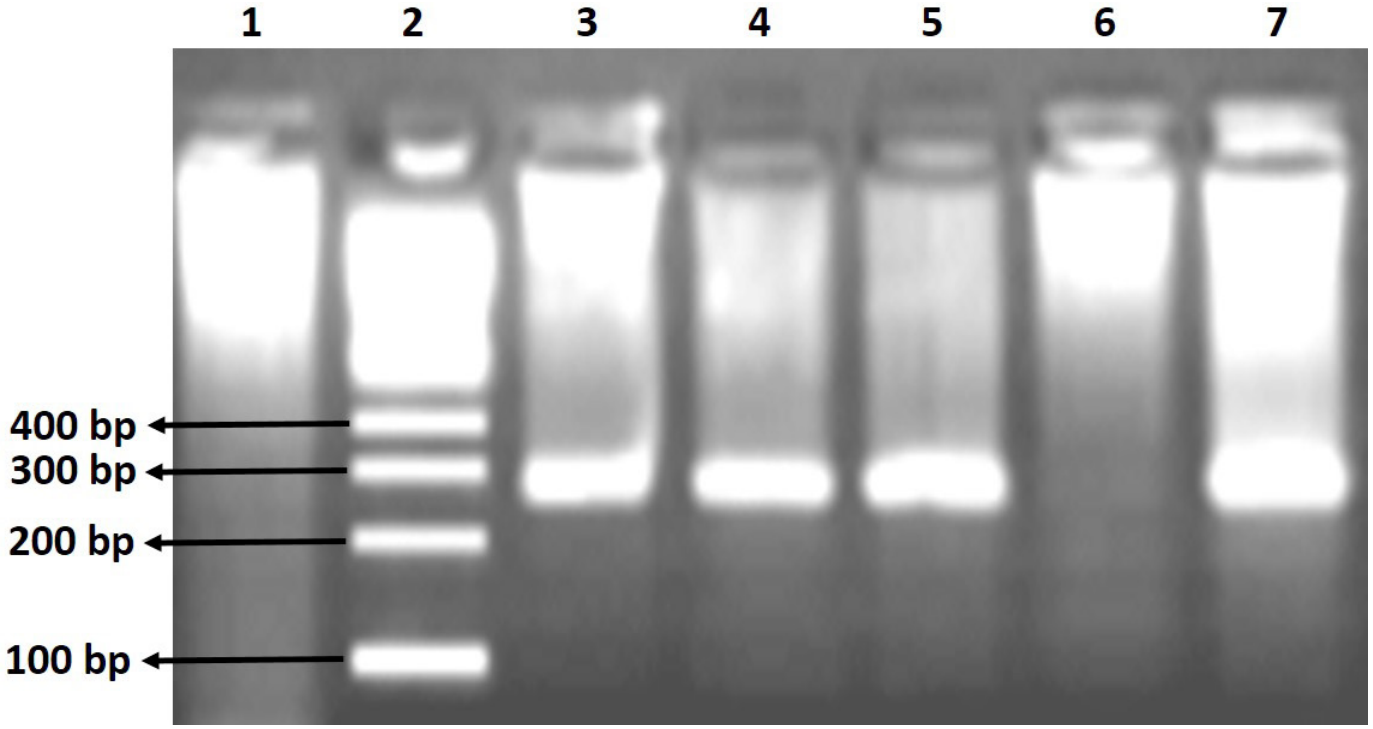

Figure 1: *Gel photograph of Brucella abortus products*
Lane 1: *Negative control;* **Lane 2:** *100-bp DNA size marker;* **Lane 3:** *Positive control;* **Lanes 4, 5 and 7:** *Positive samples;* **Lane 6:** *Negative sample*

Discussion

Real data regarding brucellosis in cattle in district Swat were not available however, Muhammad (2013) found 19.02% and 16.26% prevalence of brucellisis in cattle at District Peshawar according to SPAT and i-ELISA respectively, while at Kohat 17.58% according to SAT (Hamidullah et al., 2009). The prevalence of Brucellosis remains as a major source of disease in domesticated animals and humans worldwide. The prevalence of brucellosis differs from species to species and region to region. Other factors like climate, geographical area, density and movement of animals, genetic makeup may be responsible for this variation in prevalence.

The prevalence of brucellosis was lower in achai cattle followed by cross breed and non-descript cattle. This might be due to the fact that achai local breed of district swat is limited to the local areas. Aulakh et al. (2008) favours this statement they reported that brucellosis prevalence may differ from area to area and also vary to sex, age and species to species. It also involved various factors such as genetic makeup, geographical area, climate and density of animal kept in the area. Higher sex related prevalence varies in female might be due to production of erythritol in the pregnant animal which harbours *B. abortus*. Dinka and Regassa (2009) and Degefu et al. (2011) conducted study and reported that high prevalence was found in female cattle. Kazi et al. (2005) result were in favour that brucellosis prevalence in the old age cows is related to salinity, and the infection may latent without clinical appearance of the brucellosis.

The prevalence of brucellosis in different farming system varies; FAO-WHO (1989) survey report was in contrast with our finding that brucellosis comparatively high in intensive farms. The prevalence might be increase with the induction of animals from the nomadic to extensive and intensive farming system. Blood et al. (1979) results were in agreement to our finding who suggested that the induction of unvaccinated cattle in the herd, increasing herd size, dense population may increase the prevalence of the disease. The high infection rate of bovine brucellosis might be due to rearing of cattle in dense population. The high prevalence in nomadic system might be large herd size, free grazing and insanitary practice in field. Silva et al. (2000) and Degefu et al. (2011) were parallel to our finding they suggested that the prevalence of brucellosis in both nomadic and extensive system due to free access of animals and direct contact during grazing and watering.

Cooper (1992) also observed raw milk consumption as a high risk factor for bovine brucellosis. Direct association with household animals and consumption of unpasteurized milk and meat of animal source were the major risk factors as reported by Alballa (1995). Salari et al. (2003) reported that human may acquire infection through direct contact with diseased animals, their products, ingestion of unpasteurized milk and meat and inhalation.

Conclusions

This study suggests that SPAT, ELISA and PCR may be used for diagnosis of brucellosis with equal reliability under field conditions of the low income farming community. Abattoir shops provide a higher risk to the public health than the farms and livestock markets. Nomads herds were highly infected with brucellosis followed by intensive and extensive farming and artificial insemination exerted a greater risk as compared to natural service. The highest incidence of brucellosis found in butchers (20.58%), veterinary assistants (14.28%), farm workers (12.28%), abattoir workers (11.53%) and farm owners (10.41%) is quite alarming and needs immediate biorisk management measures by the relevant public health and local government authorities.

Authors' Contribution

Inamullah Khan: Master degree scholar; Muhammad Subhan Qureshi lead scientist and postgraduate student supervisor; Rajwali Khan supported lab work; Syed Muhammad Sohail supporting data analysis; Ijaz Ahmad supported literature searching; Muhammad Shoaib supported lab work and Asim Ijaz supported field work.

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