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Spatial Distribution of Soil Borne *Brucella* Species Specific DNA in Punjab, Pakistan

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ABSTRACT

Brucella species are cause of abortions and other reproductive disorders in animals and human beings. In this project, prevalence and distribution of soil borne *Brucella* species in nine districts of Punjab including Lahore, Faisalabad, Sheikhupura, Sargodha, D.G. Khan, Chakwal, Sahiwal, Gujranwala and Attock were determined. By using grid based sampling strategy, soil samples (n= 1280) were collected from villages (n=256, 5 samples/village) of the nine districts. *Brucella* genus specific DNA (*bcsp31*) was detected using Real Time PCR and prevalence was 2.11%. Out of five species, only two species *B. abortus* and *B. melitensis* were detected through conventioneal multiplex PCR. Prevalence of soil borne species specific DNA of *B. abortus* and *B. melitensis* was 1.8% and 0.31%, respectively. Out of nine, three districts were positive for *Brucella* species specific DNA. All districts showed a significant result for detecting *Brucella* species (χ^2=54.505, df=8, p<0.05, 95% CI). In conclusion, distribution pattern of the soil borne *Brucella* species in three districts of Punjab puts both human and animal population at a high risk of exposure. Further studies are required to explore molecular diversity of the pathogens together with sero-conversion in animals and humans.

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Authors' Contribution
RA designed the study, performed experimental work and wrote the manuscript. MR provided funds. KM supervised the study and helped in data analysis. MSK contributed in sample collections.

Key words
Brucella, DNA, Prevalence, Real-Time PCR, Soil.

INTRODUCTION

Brucellosis is a wide spread zoonosis in world and is caused by various species of *Brucella*. It is the second major zoonotic disease after rabies (OIE, 2005). The organism is categorized as Risk group III pathogen by World Health Organization (WHO) and category B pathogen by Centre of Disease Control and Prevention (CDC). Its species are intracellular facultative pathogens and mostly affect the reticulo-endothelial and reproductive system (Jarvis *et al*., 2002). It grows slowly on nutrient media and their growth can be enhanced by the addition of serum or blood. *Brucella* replicate inside cells and evade the innate and adaptive immunity and cause the disease (Ficht, 2003).

Lab workers easily acquire *Brucella* infection. It mainly affects slaughterhouse workers, butchers, and veterinarians. Humans are infected by occupational exposure, oral, respiratory and conjunctival routes but ingestion of unpasteurized dairy milk is the main risk of getting *Brucella* infection. Signs of disease in humans are undulant fever, musculo–skeletal, cardiovascular, central nervous systems complications, arthritis, orchitis and epididymitis (Palmer *et al*. 1998).

Risk factors for brucellosis are herd type, flooring system, ventilation, housing conditions, rented pasture to feed cattle and presence of wetland on farm (Borba *et al*., 2013).

Brucellosis is important disease that also affect economics of the livestock farmers in the developing countries (Bernues *et al*., 1997) The component for calculating economic loss were loss due to reduction in meat yield by abortion, still birth, repeat breeding, veterinary expenses and infertility (Radostisis *et al*., 2000).

In Pakistan, sheep and goats (small ruminants) are reported for prevalence of the disease by many authors and it was found to be 13.5% to 42.6% (Iqbal *et al*., 2013; Mirza *et al*., 1998), in large ruminants (Cattle and Buffalo) 3.84% to 47.19% (Soomro *et al*., 2014; Nasir *et al*., 1999) and in human beings was found to be 6.79% to 6.9% (Ali *et al*., 2013).

Soil provides the complex habitat for the microorganisms but their number is very high in surface soil around the macropores (Bundt *et al*., 2001). The macropores are the channels in the soil which are formed by the activities of earthworms, roots of the plants and other soil biota which is lined with organic matter mostly in top soil (Fierer *et al*., 2007). The bacterial growth and diversity is correlated with organic matter. The microbial diversity and number is very high in top 10 cm of the soil and decreases with the depth (Eilers *et al*., 2012). It is estimated that number of species of bacteria in the soil

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ranges from 2000 to 18,000 per gram (Ritz *et al*., 2003).

Brucella species are sensitive to heat and can survive for several weeks in water (Franz *et al*., 1997). *Brucella* species survive in the moist soil for a long period and can be transferred to relevant host (Kuzdas and Morse, 1954). *Brucella* species survive in soil and dust for many weeks (Franz *et al*., 1997). *B. abortus* can survive on vegetation, soil and fetal tissues for several weeks depending month, temperature and sunlight (Aune *et al*., 2012). *B. abortus* survive up to 135 days (in aborted fetus), more than 60 days (in cool environment) (Aune et al., 2012) and up to six months (in shaded fetus) (Wray, 1975). Sunlight and temperature has impact on survival of B. *abortus* in the environment (Jones *et al*., 2010). *B. abortus* can survive up to 66 days in wet soil, when humidity increased up to 90%, it can survive for 48 to 73 days and less than 4 days in dry soil (Nicoletti, 2001). *B. suis* survive up to 28 days at 5^0C or 22^0C (Worth and Wendling, 2012).

Livestock sector contributes 11.9% share in GDP of Pakistan (Amjid *et al*., 2011). Most of the people earn from livestock. A lot of studies have been done on serological prevalence of brucellosis but no study has been done on the prevalence of soil borne *Brucella* species in Pakistan. The persistence of *Brucella* species in soil (Nicoletti, 2001) is a great risk for animals and humans health.

Therefore present study is designed to investigate the persistence of soil borne *Brucella* species and to achieve the following objectives: (i) detection of soil borne *Brucella* genus specific DNA by using Real time PCR and species specific DNA by conventional Multiplex PCR and (ii) mapping of distribution of *Brucella* species in various villages of Punjab.

MATERIALS AND METHODS

Study area and sample collection

The present study was designed to determine spatial distribution of soil borne *Brucella* species (*B. abortus*, *B. melitensis*, *B. suis*, *B. ovis* and *B. canis*) in Punjab Pakistan. The soil samples were collected from nine districts of Punjab. To select the district for sampling, first of all a map of Punjab was scanned on 600 dpi resolution and geo-referenced with geographic coordinate system. The boundary line of the study area was marked through GPS survey using the ground control points. Other layers including roads, canals, drains and rivers were digitized from Landsat-ETM downloaded from the USGS website (Free source). All of the layers were overlaid and used to make multivariate map. To determine risk areas according to intensity of *Brucella* species, the geographical mapping of the study area for the soil characteristics, animal and human population with respect to prevalence of pathogen was generated. Furthermore, the maps were generated in the same way district wise of all the districts. The layers including roads, canals, drains and rivers in maps were generated from survey of Pakistan districts maps. Inset map was generated from University of the Punjab, Lahore, Pakistan. All the geographical coordinates of the samples were recorded using GPS receiver (Garmin, Dakota U.S.A.) and mapped using recorded data and sequence. ArcGIS 10.1 (Esri, California) software was used to develop maps to see the spatial distribution of *Brucella* species in nine districts (Gujranwala, Faisalabad, Sargodha, Sahiwal, D. G. Khan, Chakwal, Sheikhupura, Lahore and Attock).

The soil samples (n=1280) from 10 % villages (n= 256 villages) of nine districts of Punjab including Sargodha, Sahiwal, D.G. Khan, Chakwal, Attock, Sheikhupura, Gujranwala, Faisalabad and Lahore were collected [From each district, 10% of total villages were randomly selected using OpenEpi version 2.3.1 (http://www.openepi.com/OE2.3/Menu/OpenEpiMenu.htm) software using a confidence limit (5%), level of significance 80%] (Fig. 1).

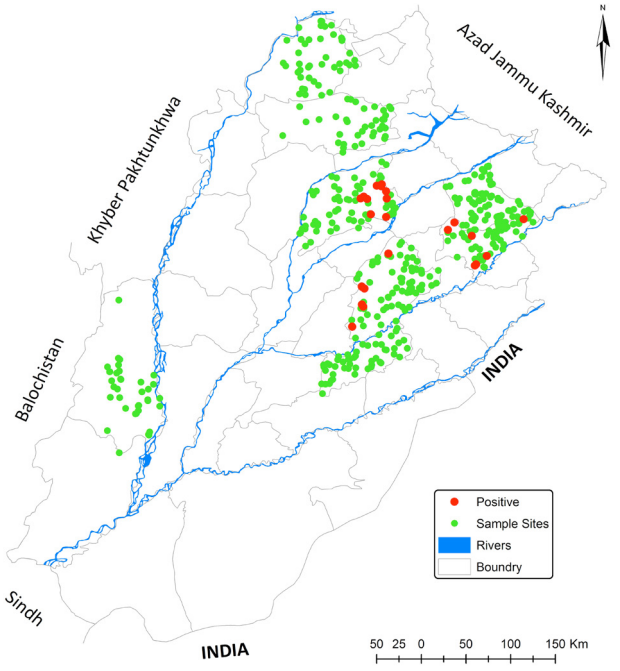

Fig. 1. Geospatial distribution of *Brucella* genus specific DNA in soil samples collected from nine districts of Punjab.

Personal protective equipments (PPEs) were used for soil sampling. Samples were collected by using portable electronic weighing balance and placed in pre-labeled clean polythene bags. From each village five samples were collected. Samples were collected in such a way that four

samples (n=4) were taken from each corner of the village where animals and humans were in close proximity while the fifth sample was taken as a control from outside the village, the soil where human and animal populations were not frequently interacting. The soil samples were taken three inches below the ground surface. The soil quantity was taken as 500g, 500g and 250 g and then these were pooled by mixing. One sample about 750g was archived for future reference and second sample (500g) was processed for DNA extraction in University Diagnostic Laboratory (UDL), UVAS Lahore.

A total of 130 soil samples were collected from 26 villages of Lahore. Total 145, 155, 145, 185, 135, 130, 125 and 130 soil samples were collected from 29 villages of Sheikhupura, 31 villages of Faisalabad, 29 villages of Sargodha, 37 villages of Sahiwal, 27 villages of D.G. Khan, 26 villages of Chakwal, 25 villages of Attock and 26 villages of Gujranwala, respectively.

DNA extraction from soil samples

About 0.25 g of soil sample was measured through weighing balance and labelled with proper code. DNA was extracted from the soil samples using PowerMax™ Soil DNA Isolation Kit as per manufacturer's recommendations. DNA quality and quantity was determined using Nano Drop 1000 spectrophotometer (Nano Drop, USA) and Qubit flourometer (Invitrogen, USA) using DNA BR assay kit (Invitrogen, USA) as per manufacturer's instructions.

Real-Time PCR

The prevalence of soil borne *Brucella* genus specific DNA was detected using RT PCR (CFX96™ Real-Time PCR Detection Machine BIO-RAD, U.S.A). A 223 bp fragment of *bcsp-31 gene was amplified by RT-PCR*. For the amplification, Forward Primer 5'-GCTCGGTTGCCAATATCAATGC-3', Reverse Primer 5'-GGGTAAAGCGTCGCCAGAAG-3' and Probe 6-FAM-AAATCTTCCACCTTGCCCTTGCCATCA-BHQ1 was used. Real time-PCR was optimized for detection of soil borne genus *Brucella* using extracted genome from soil samples. The reaction was optimized for standard concentrations of primers, probe and DNA. The known DNA standards were provided by Friedrich Loeffler Institute (FLI), Germany. The concentration of Known DNA was 100pg/µL. About three different concentrations of Forward primer, Reverse primer and probe like 10, 20 and 30 *pmoles* per reaction were used, respectively. Along with primers and probe, about four different concentrations (10, 15, 20 and 30 ng/reaction) of DNA were used for optimization. PCR conditions and reaction mixture composition was followed as described by Probert *et al*. (2004).

Conventional multiplex PCR

The soil DNA samples which were positive for genus *Brucella* in real-time PCR were again analyzed in conventional Multiplex PCR for detection of *Brucella* species. The species specific primers were used to detect species like *B. abortus* by targeting gene *IS711* (498 bp) using FP 5' GACGAACGGAATTTTTCCAATCCC-3', RP 5' TGCCGATCACTTAAGGGCCTTCAT-3', *B. melitensis* gene *IS711* (731bp) using FP 5' AAATCGCGTCCTTGCTGGTCTGA-3', RP 5'-TGCCGATCACTTAAGGGCCTTCAT-3', *B. suis* by targeting *IS711* gene (286 bp) using FP 5'- GCGCGGTTTTCTGAAGGTTCAGG-3', RP 5'- TGCCGATCACTTAAGGGCCTTCAT-3', *B. ovis* by gene *IS711* (976 bp) with FP 5'-CGGGTTCTGGCACCATCGTCG-3', RP 5' TGCCGATCACTTAAGGGCCTTCAT-3'and *B. canis* by targeting gene *IS711* (276 bp) using FP 5'- CCAGTATAGCTGGGCTGGTG-3', RP 5'-CGGATATCCTGCGTGTCCAG-3'through conventional Multiplex PCR. The PCR conditions, composition of reaction mixture and agarose gel electrophoresis was performed and followed according to Ali *et al*. (2014).

Data analysis

The results of real-time PCR and conventional Multiplex PCR analysis were compiled in a single Microsoft Excel spreadsheet. Data was analyzed through chi square test using 95% confidence interval and 5% level of significance. A statistical software SPSS (version 20.0; SPSS Inc., Chicago, IL) was used for statistical analysis. Normality of data was also checked by using Shapiro-wilk test.

RESULTS

Out of total 1280 soil samples collected from 256 villages (10% villages of each district) of the nine districts of Punjab when processed for the detection of *Brucella* genus specific DNA using genus specific primers and probes through RT-PCR, 27 samples (2.11%) were declared as positive. Threshold cycle (Ct) value was above than threshold for detection of genus *Brucella* DNA. Out of nine districts, three districts Sargodha, Sheikhupura and Faisalabad were positive for *Brucella* genus specific DNA. In district Faisalabad, out of 155 soil samples collected from 31 villages when processed through RT-PCR, nine (5.80%) samples were positive (Fig. 2), in district Sargodha, out of 145 soil samples collected from 29 villages, eleven (7.58%) samples were positive (Fig. 3) and in district Sheikhupura, out of 145 soil samples collected from 29 villages when processed through real-time PCR, seven (4.82%) samples were positive for *Brucella* genus specific DNA (Fig. 4).

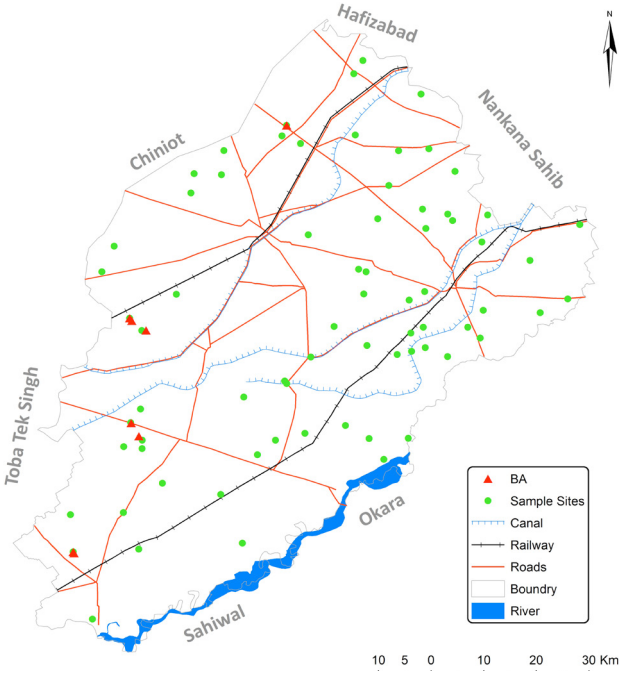

Fig. 2. Geospatial distribution of *B. abortus* DNA in soil samples collected in Faisalabad.

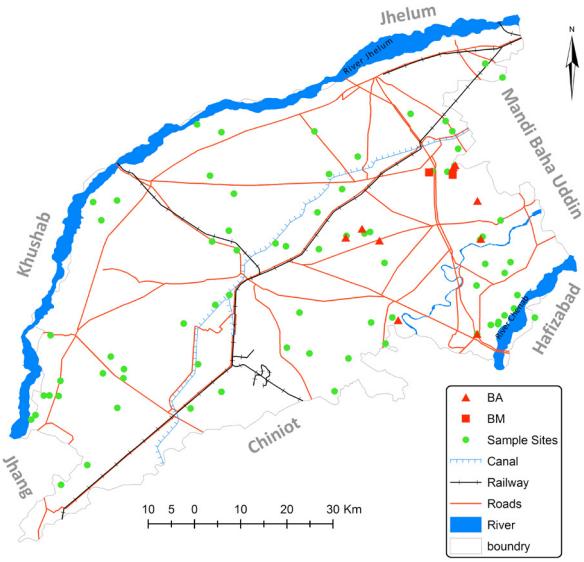

Fig. 3. Geospatial distribution of *B. abortus* and *B. melitensis* DNA in soil samples collected in Sargodha district, Pakistan

Soil samples which were positive for *Brucella* genus specific DNA through RT-PCR, were again run on Conventional Multiplex PCR in order to find out the exact *Brucella* specie by using species specific primers. Through conventional multiplex PCR, out of five species (*B. abortus, B. melitensis, B. suis, B. ovis* and *B. canis*) only two species *B. abortus* and *B. melitensis* were detected. Out of 1280 soil samples, 23 (1.8%) soil samples were positive for *B. abortus* DNA while four (0.31%) soil samples were positive for species specific DNA of *B. melitensis*. PCR product was run on 2% agarose gel, and bands for *B. abortus* (498bp) and for *B. melitensis* (731bp) were seen on gel.

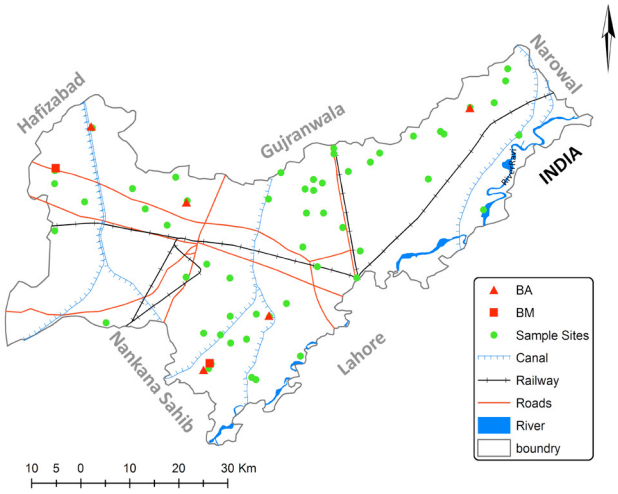

Fig. 4. Geospatial distribution of *B. abortus* and *B. melitensis* DNA in soil samples collected in Sheikhupura district, Pakistan

Out of nine districts, three (33.33%) districts Sheikhupura, Faisalabad and Sargodha were positive for soil borne *B. abortus* DNA and two (22.22%) districts Sheikhupura and Sargodha were positive for *B. melitensis* DNA. Out of 29 tehsils, 9 (31.03%) were positive for *B. abortus* DNA and three (10.34%) were positive for *B. melitensis* DNA. Tehsil wise *Brucella* genus specific DNA was detected significantly (χ^2 =75.977, df=32, p<0.05, 95% CI), *B. abortus* DNA was also detected significantly (χ^2 =76.805, df=32, p<0.05, 95% CI) but soil borne *B. melitensis* DNA did not show significant result (χ^2 =40.128, df=32, p>0.05, 95% CI). Out of 196 union councils, 18 (9.18%) were positive for *Brucella* genus specific DNA, 14 (7.14%) were positive for *B. abortus* DNA and four (2.04%) union councils were positive for *B. melitensis* DNA. In union councils, soil borne *Brucella* genus specific DNA (χ^2 =330.334, df=202, p<0.05, 95% CI) and *B. abortus* species specific DNA (χ^2 =326.052, df=202, p<0.05, 95% CI) were detected significantly but *B. melitensis* DNA (χ^2 =187.839, df=202, p>0.05, 95% CI) was not detected significantly. Out of 256 villages, 19 (7.42%) were positive for *B. abortus* DNA and four (1.56%) were positive for *B. melitensis* DNA. village wise

detection of *B. abortus* (χ^2=297.937, df=308, p>0.05, 95% CI) and *B. melitensis* DNA (χ^2=268.840, df=308, p>0.05, 95% CI) was not significant.

In district Sheikhupura, out of 145 soil samples collected from 29 villages, when processed through Conventional multiplex PCR, five (3.44%) samples were positive for *B. abortus* DNA while two (1.38%) samples were positive for *B. melitensis* DNA. In district Sheikhupura three tehsils like Sharqpur, Mureedkey and Ferozwala were positive for *B. abortus* DNA while two tehsils like Sheikhupura and Ferozwala were positive for *B. melitensis* DNA. Five (17.24%) villages like Abdalia, Mirza virkan, Muradpur, Kilay and Thatta Alyas were positive for *B. abortus* DNA and two (6.89%) villages like Chambal and Chak Phannar were found positive for *B. melitensis* DNA.

In district Faisalabad, out of 155 soil samples collected from 31 villages, when processed through Conventional multiplex PCR, nine (5.80%) samples were positive for *B. abortus* DNA. None of the samples was found positive for *B. melitensis* DNA in this district.

Four tehsils in district Faisalabad like Chak jhumra, Samandri, Tandlianwala and Faisalabad were positive for *B. abortus* DNA. Six (19.35%) villages like Chak 106, Chak 142 GB, Chak 228 GB, Chak 507 GB, Chak 86 JB and Chak 88 JB were positive for *B. abortus* DNA.

In district Sargodha, out of the 145 soil samples collected from 29 villages when processed through Conventional multiplex PCR, nine (6.20%) samples were positive for *B. abortus* DNA while two (1.38%) samples were positive for *B. melitensis* DNA. Three tehsils in district Sargodha like Bhalwal, Kot Momin and Sargodha were found positive for *B. abortus* DNA and one tehsil Kot Momin was found positive for *B. melitensis* DNA. Eight (27.58%) villages like Moazamabad, Chak 15/sb, Chak 16/sb, Chak Khana, Rawana, Chanidal, Chak 3/sb and Laluwali were positive for *B. abortus* DNA while two (6.89%) villages like Dera madansani and Abdal were found positive for *B. melitensis* DNA.

Out of the 130 soil samples collected from 26 villages of Gujranwala district, 130 soil samples from 26 villages of Lahore district, 185 soil samples from 37 villages of Sahiwal district, 135 soil samples from 27 villages of D.G. Khan District, 130 soil samples from 26 villages of Chakwal district and 125 soil samples were collected from 25 villages of Attock district when processed through conventional multiplex PCR, none of the samples was found positive for species specific DNA of *B. abortus* and *B. melitensis*.

DISCUSSION

Brucella species are mostly diagnosed in the laboratory by serological tests which are easy to perform but these tests lack specificity and *Brucella* species cross react with other bacteria particularly *Yersinia enterocolitica* O:9 that results due to O chain antigenic similarity (Young, 1991; Wrathall *et al*., 1993; Garin-Bastuji *et al*., 1999; Godfroid *et al*., 2002; Nielsen *et al*., 2004). Therefore, diagnosis through culturing is "gold standard" but due to the zoonotic nature of *Brucella* species, it poses a great potential hazard to laboratory workers and culturing should be done in well-equipped lab (Biosafety level 3) with highly skilled personnel. In the present study, real-Time PCR is used and *Brucella* genus specific DNA was detected by targeting *bcsp* 31 gene. Real-time PCR is used because it is highly specific and sensitive (97%), do not need high biosafety level 3, poses less threat to humans, simultaneous detection of multiple samples and rapid turnaround time.

DNA from bacterial cells can be released into the environment either by active secretion or passively after their death. There is autolysis of dead bacterial cells and release of cytoplasmic contents including DNA (Palmen and Hellingwerf, 1995). Other bacteria have access towards DNA of lysed cells (Nielsen *et al*., 2000). A significant amount of DNA is released from bacterial cells when they have lethal exposure to antibacterial agents (Friedlander, 1975). Many genera including *Acinetobacter, Alcaligenes, Azotobacter, Bacillus, Flavobacterium, Micrococcus, Neisseria* and *Pseudomonas* release DNA during active growth (Dillard and Seifert, 2001; Hamilton *et al*., 2005; Lorenz *et al*., 1991; Lorenz and Wackernagel, 1994; Paget and Simonet, 1994; Thomas and Nielsen, 2005; Yin and Stotzky, 1997). *Escherichia coli* also release extracellular plasmid DNA during co-cultivation with algae (Matsui *et al*., 2003). *Deinococcus radiodurans* actively excrete its DNA after damage with U.V. light (Battista, 1997).

In the present study soil samples were collected from the four corners of the villages where animals were kept in close contact with humans and one sample was collected from the soil which was not inhabited by either animals or humans. Survival of the organisms in the environment poses a great health risk to animals and humans. Soil is an enriched environment with nucleic acid that is consider to be mostly present in upper soil layers (Baker, 1977). Regarding the DNA presence in the soil, it was estimated that about 70% DNA present in upper soil is of fungal origin (Borneman and Hartin, 2000; Smit *et al*., 1999; van Elsas *et al*., 2000). According to Niemeyer and Gessler (2002), from one gram of the soil, 80 µg DNA can be extracted. DNA in the soil is rapidly hydrolyzed by nucleases (Greaves and Wilson, 1969; Romanowski *et al*., 1992; Widmer *et al*., 1996) and majority of nuclease activity in the soil is of bacterial origin (Blum *et al*., 1997).

In the present study, the soil of districts Sargodha, Faisalabad and Sheikhupura is positive for genus Brucella species specific DNA. Sargodha City is located in longitude 72° 38′ to 72° 43′ and latitude of 32° 3′ to 32° 7′ and is situated at a distance of about 185 km towards north-west of Lahore. The city is linked with Faisalabad, Jhang, Sheikhupura, Khushab, Gujrat and Sialkot by metaled roads. Total area covered by the city is 5854 KM^2 and 3.1 million human population. Two rivers, river Jhelum and river Chenab flows near the city (Fig. 3). Minimum temperature of the city is as low as freezing point in winter and in summer the temperature reaches up to 50°C (122 °F). District Sargodha showed positive soil samples for *Brucella* species specific DNA as the soil of this district is of sandy loam type and chances of DNA survival and stability are very high in this type of soil as described by Selenska and Klingmüller (1992).

District Faisalabad is located with latitude 31.3235° N and longitude 73.1822° E and it is situated at elevation 186 meters above sea level and covers an area of 1269 Km^2 with human population of 2.5 million, making it the 2nd biggest city in Punjab (Fig. 2). In Faisalabad districts, the positive soil of tehsils Chak Jhumra, Tandlianwala and Samandari is silty, clay and sandy type and this soil supports DNA stability up to months as described by Romanowski *et al*. (1993).

District Sheikhupura is located with latitude 31.7166^{0}N and longitude 73.9850^{0}E. The district covers 5 tehsils, 169 union councils and total land area of 5960 Km^2 with human population of 0.28 million (Sathar and Casterline, 1998). It is situated at 237m high from sea level and 16th largest city of Pakistan (Fig. 4). The positive soil of tehsils Sharqpur, Mureedkey and Ferozwala of district Sheikhupura is loamy sand type and according to Recorbet *et al*. (1993) this type of soil supports DNA stability up to months. The contaminated soil by *Brucella* species (which are excreted through vaginal discharge, aborted fetuses, milk and semen of infected animals) is the source of infection to humans through parenteral, by air droplets, contact and through oral-fecal route (La Placa, 2010). The organism is highly infectious and cab be aerosolized and very difficult to diagnose due to non-specific symptoms associated with its infection (Doganay and Doganay, 2013).

B. abortus survives in aborted fetuses, manure and water for periods of 150 to 240 days (Saegerman *et al*., 2011). There is long term survival of DNA in the environment from archaeological and paleontological remains (DeSalle *et al*., 1992; Hofreiter *et al*., 2001; Smith *et al*., 2001). DNA has been amplified from thousands of years old specimens through PCR (Austin *et al*., 1997; Landweber, 1999). DNA resist environmental conditions and survive for a long time and is prevented from degradation by Nucleases. The survival of *Brucella* species in the environment is a great risk for humans and animals health. Organism can survive up to 122 days at room temperature in cow feces and up to 10 days in aquatic environment at 25^{0}C (Mitscherlich and Marth, 2012). The lab workers, veterinary doctors, butchers, veterinary technicians, insemination service employees, zoo technicians, farmers working on multi-herd farms, employees of meat and milk processing enterprises are at great risk of exposure with *Brucella* species (Galinska and Zagórski, 2013). Infected people show the symptoms of fever, arthralgia, myalgia, back pain, hepatomegaly, splenomegaly, endocarditis, neurobrucellosis, epididymitis and orchitis (Dean *et al*., 2012; Godfroid *et al*., 2011; Olsen and Palmer, 2014).

In the present study, overall 2.1 % prevalence of *Brucella* species was observed while 12.4% was observed in Argentina (Samartino, 2002), 15.9 % in Brazil (Borba *et al*., 2013), 8.5 % in Georgia (Mamisashvili *et al*., 2013), 26.3 % in Algeria (Aggad and Boukraa, 2006), 20.3 % in Cameroon (Scolamacchia *et al*., 2010), 4.98 % in Egypt (Samaha *et al*., 2008), 20.4 % in Ethiopia (Mekonnen *et al*., 2010), 42.2 % in Libya (Ahmed *et al*., 2010), 14.2 % in Niger (Boukary *et al*., 2013), 77.5 % in Nigeria (Mai *et al*., 2012), 56.4 % in Zambia (Muma *et al*., 2007), 3.7 % in Iran (Akbarmehr and Ghiyamirad, 2011), 25.8 % in Jordan (Al-Majali *et al*., 2009), 12.0 % in Kyrgyzstan (Bonfoh *et al*., 2012), 4.1 % in Tajikistan (Lindahl *et al*., 2014), 35.3 % in Turkey (Şahin *et al*., 2008) and 13.6 % in India (Kumar *et al*., 2005; Trangadia *et al*., 2010). *Brucella* species are not detected in districts Lahore, D.G. Khan, Chakwal, Sahiwal, Gujranwala and Attock. The possible reasons for not detecting DNAs may be (a) there is low animal density (b) less number of households (c) high nucleases activity in the soil (d) exposure to high temperature (Bauer *et al*., 2003).

CONCLUSION

In conclusion, DNA of *B. abortus* and *B. melitensis* is distributed in the soil of districts Faisalabad, Sheikhupura and Sargodha. This pattern of distribution of *Brucella* species specific DNA in the districts of Punjab puts both human and animal population at a high risk of exposure. Further studies are required to explore molecular diversity (biovars) of the pathogens together with sero-conversion in animals and humans.

Statement of conflict of interest

Authors have declared no conflict of interest.

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