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Serological Detection, Risk Factor Analysts and Statistical Mapping of Brucellosts in Females of KP Pakistan

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Abstract. Among the most well-known zoonotic maladies, Brucellosis is the one having a lot of clinical presentations. Brucellosis is a disease which is caused by a type of bacteria of the genus Brucella. Brucellosis is common in people who work as shepherd, milkman, butcher, knackers and vertinary assistants. The different types of the tests are used to diagnose brucellosis such as (ELISA) Enzyme Linked Immunosorbent Assay, (RBPT) Rose Bengal Plate Test and many others. From literature it is evident that the picture of brucellosis is not clear in many countries including Pakistan. So the aim of the study is to clear the exact status of the disease in the women of agro ecological zones of KP Pakistan. A multistage sampling will be used by dividing KP in different agro ecological zones and blood will be collected from the women who are in close contact with animals. The serum will be isolated and the samples will be tested for Brucella by commercial serological kits i.e. ELISA kit in the laboratory for the comparison of the disease status in women of different agro ecological zones of KP Pakistan.

Keywords: Serological Detection, Statistical Mapping, Brucellosis, Bacteria, ELISA.

1. Introduction

Among the widespread diseases in the world, Brucellosis is the one transmittable disease (Papas, Papadimitriu et al., 2006), and is caused by Brucella (Godfroid, Scholz et al., 2011) and are responsible for the pathogenesis of brucellosis and have the power invade phagocytic cells and can multiply rapidly inside the cells (Jarvis et al., 2002). Six classic Brucella species have been recorded since the 1960s, and the range of recognized Brucella species has slightly doubled since earlier times (Pappas 2010). British surgeon David Bruce first discovered brucellosis in 1887 (Bakri et al., 2018). He identified a type of gram negative bacteria from the bodies of British soldiers who died in Malta because of fever. Brucellosis has many synonyms or derivatives on the basis of the disease type or its distribution or may also by the resemblance with diseases like typhoid fever, malaria, physical disorders and Maltese fever (Madkour 2001). The genus Brucella includes many different types of species on basis of its host and many other causes. The different species include Brucella neotomae in desert rat, Brucella suis cause disease in pigs, reindeer and rabbit, Brucella melitensis in sheep and goats, Brucella canis in dogs and likewise many other species which are more common in different parts of the world including Africa, Asia America and parts of Middle East (Corbel 2006). Brucellosis infects all the mammals including human and many other animals (Cutler et al. 2005). Brucellosis is the most advanced and undiagnosed infection worldwide, as well as in Pakistan (Rubach et al., 2013; WHO, 2012). According to (Perugini, Capuano et al., 2009) brucellosis is considered as an animal crime that cause great harm to animals. It is considered the second most important zoonosis in the world (Perugini, Capuano et al. 2009). Variances in host preferences for a given species are currently being achieved through differential experiments aimed at elucidating the phenotype of lipopolysaccharide (LPS) antigens, phage invasion, color identification, production requirements of CO2, H2S production and attention to metabolic components (Perugini Capuano et al. 2009). Brucella lives and reproduces in the host's macrophages which are beneficial for self- defense. Over time, brucellosis separates from white blood cells in the different organs of the body like bone marrow, spleen and many other organs. Abortion and membrane retention in bulls and cattle is the sign of Bovine brucellosis (Acha and Szyfres, 2003). The transmission can occur directly by the contact with aborted fetuses or may indirectly by the contact with infected pathogens. Intake of unpasteurized milk or other products can also play an important role (Bhat et al., 2010). Human contact with secretions, especially during childbirth and abortion can transmit the disease. There is some evidence that diseases can also transmitted through sexual contact which are extremely rare and can be transmitted by donating blood or may also by the transplantation of tissue, and even those who work in laboratory may also be at risk (Doganai and Aigen, 2003). It is difficult to diagnose brucellosis on the basis of clinical signs as it is very similar to other diseases like that of malaria and typhoid diseases. Brucellosis causes a great economic and health losses and is widespread in many countries (Bandara and Mahipale, 2002). For the clinical diagnosis of brucellosis it is important to know about the medical history of patient. Early and accurate diagnosis is essential to treat and stop the disease. Recognition is provided primarily by

culture and different diagnostic techniques which also aid in herd control, monitoring programs and planning (Dos Santos et al. 2017; Ducrotoy et al. 2018). Various laboratory methods are useful for diagnosing brucellosis, such as body isolation and identification, methods for detecting Brucella-specific antigens and antibodies and molecular detection. The Enzyme Linked immunosorbent Assay (ELISA) is the common test used to diagnose the brucellosis (Matope, Bhebhe et al. 2011). The Rose Bengal Plate Test (RBPT) is a simple and inexpensive remnant evaluation which is used as diagnostic tool for the brucellosis in small animals (Nicoletti, 1980). The highest adherence can be achieved in both sheep and goats (Thakur, Thapliyal et al. 2003). ELISA is inexpensive, sensitive, specific, and fast, which is easy to understand with colorimetric results (Roushan 2010) and is widely used as diagnostic tool for brucellosis. The control of brucellosis in animals consists of vaccinating farm animals against the disease, providing feed for animals that are healthy and free of brucellosis bacteria and implementing measures and medical protocols to guarantee it, which is the only way to control disease in humans (Ducrotoy et al., 2017). Due to the survival of brucellosis inside the cells and its ability to adapt to white blood cells (Farid et al. 1961; Seleem et al. 2008), its treatment in animals by antibiotics is not useful. Appearance of infections again and again are very common in humans. Treatment of brucellosis in humans requires careful selection of drug combinations to avoid side effects and the development of resistance (Villate and Casallas 2020). Trade in animal products is increasing around the world through which most pathogens spread rapidly. When trading and transporting livestock and animal products locally, regionally, nationally and internationally, various rules and regulations must be strictly followed. The transport of products of animal origin must be carried out by following the general principles of the International Zoological Code (Yin et al. 2024), as well as general guidelines and practices. In principle, various animal testing procedures and quarantine measures must also be followed in accordance with this regulation (OIE 2016). Brucellosis is widespread around the world and represents a serious threat to human and animal health throughout the world. It is related with great indisposition in both humans and animals in many developing countries, despite its severe impact on economic losses (Tadesse et al., 2016; Colmenero et al., 1996) and brucellosis cross-contamination (Aggad et al., 2006; Jennings et al., 2007). Brucellosis has been reported in many countries like Middle East, but neither Africa nor the Asian continent is an exception (Mussalam et al., 2016; Garofolo et al., 2016).

Brucella infection is widespread in both humans and animals in various regions of the world. Additionally, numerous studies have documented human cases of seropositive brucellosis, especially among veterinarians and breeders (Norman et al., 2016; Chen et al., 2014). Nielsen et al. (2005) reported 144 Hawaiian monk seals using the antigen plate adhesion assay (BPAT), ELISA (enzyme linked immnosorbent assay) and FPA. 164 serum samples were collected and there were no signs of clinical brucellosis in the HIV-infected animals tested. Punjab is the second biggest state area wise and has the largest human and animal population in Pakistan (Ghori et al., 2017; PBTI). Khyber Pakhtunkhwa (KPC) is the third largest state in Pakistan in terms of livestock and population. According to the 2006 census of animals, there are around 808,068 calves, 184,229 dry and 97,664 calves and around 6,059,041 females without calving, 6,059,041 females and 6,059,041 calves. A very high prevalence of 17.58% was found in the Cohart livestock area, but a low prevalence of 3.97% was reported in two districts, Bannu and Lakki Marwat. (Hamidullah et al., 2009; Bakhtullah et al., 2014) A study of buffalo brucellosis showed an incidence of 11% in the Swat Valley of KPK territory (khana et al., 2017). A total of 278 serum samples and 212 milk samples were collected in order to show status of brucellosis in small ruminants by Ali et al., (2014). Diagnostic tests used were RBPT and MRT. RBPT showed 8.6% of positive serum samples and MRT showed 9.4% positive milk samples. Overall prevalence of brucellosis in Pakistan reported was 3.25 to 4.4% among farm animals. According to SAT, the value of prevalence 5.06% and 5.49% respectively in goats and sheep. The prevalence of brucellosis in goats and sheep was 1.94% and 1.47%, respectively (Nasir et al., 2000). Pakistan has a population of over 27.8 million and producing 54,328,000 tons of milk per year (Pasha & Hafiz, 2017; ESOP, 2014, Iqbal & Ashaf, 2017) with a livestock population of 186.2 million. In addition, some 3, 0 to 8 million households have livestock-based livelihoods, most of them in rural Pakistan. Livestock production contributes significantly to agriculture around 60% and 11.2% of Pakistan's GDP (Jan and Akram, 2018; GoP, 2019; Chandio et al., 2017). Person in direct contact with animals have a greater chances of brucellosis as compare to other people and professionals. Veterinarians by the contact through vaginal secretions, uterine fluid, embryos, abortion, blood, urine and feces from infected animals or through the skin can become ill with brucellosis (Corbel 2006, Saddique et al., 2019).

Human exposure to brucellosis was found in 7 of the 29 articles reviewed, and all studies show occupational hazards. The prevalence of brucellosis was found to range from 5.8% to 17.74% in different regions of Punjab, Pakistan (Ali et al., 2016; Shehzad et al., 2020). The incidence of brucellosis is higher in butchers (27.1%) compared to scavengers (18%) and drivers (0%) (Mukhtar, 2010) in Lahore, Pakistan. In Pakistan, the disease is more common as rural women are more involved in working with animals and their products (Abubakar et al., 2011). Hussein et al., (2008) conducted a study of brucellosis in people such as villagers, ranchers and slaughterhouse workers. In this study the researchers recorded an overall prevalence of brucella in humans to be 14%. Ali et al.,

2016 found that brucellosis is more common in rural than urban women, and that Brucella infected women have symptoms and a history of stillbirth, miscarriage and endometrial miscarriage. Niaz et al. (2020) calculated the brucellosis status of a person in the Malakand region, Pakistan and noted the global ratio by age was 18.42%. Saddique et al., (2019) estimated around 10.1% of Brucella abortus infections in humans in Rawalpindi, Pakistan. The incidence of Brucella infection was higher in men and women who had contact with animals for at least 40 years. In a similar study, Ali et al. (2018) recorded a high prevalence in the people of the age 20 to 30 years and found that the prevalence was higher among those who live in rural areas and have animals at home and are ignorant. Based on the previous study, although it has been studied extensively in animals such as cattle, buffalo, dogs, and horses in many developing countries, including Pakistan, the outlook for brucellosis in humans remains unclear (Yesuf et al., 2010).

Hence objectives of the study is to serologically demonstrate brucellosis and show the exact incidence status of women from different agroecological regions of KP, Pakistan.

2. Objective of This Study

1. Determination of the prevalence and risk factors of brucellosis by ELISA in various agroecological regions of KP Pakistan.
2. Comparison of different KP ecological regions of Pakistan with respect to different climatic conditions such as temperature, precipitation and atmospheric pressure.

3. Research Methodology

3.1. Ethical Approval

The study approved by the Department of Zoology, Abdul Wali Khan University Mardan.

3.2. Study Area

Prevalence of brucellosis has been observed in various agroecological areas in five districts of KP Pakistan. These districts are Mardan, Dir, Mohmand Agency, Kohat, and Mansehra. Mardan is located in the southwestern part of the region at an elevation of 34° 12'0 N, 72° 1'60 E, and 283 m (928ft) and is characterized by a warm semi-arid climate. Dir is in the northwest approximately 35° 10'N 72° 00 E. Altitude 1420m and humid subtropical climate. Mohmand Agency is an area in the Peshawar area at 34° 30'00 "N and 71° 20'00" E and has an elevation of 19.94 meters (2362feet). Mohmand's Agency climate is hot in summer and cool in winter. The winter season begins in November and lasts until February. There is little rain here. Most of the precipitation falls in winter. Kohat is in coordinates of 33°35N 71°26E. Its height is 489 meters (1604 feet). The climate in Kohat is warm and semi-arid. Mansehra is located at 34° 19'59 "N and 73° 12'00" E, and is 1088m=3569 feet above sea level. Mansehra has oceanic climate. It rains all year round. The average annual temperature in Mansehra is 23 degrees and the annual rainfall is 601mm. There are 183 dry days a year with an average humidity of 43%.

3.3. Samples Collection

A multi-stage sampling was used from women from different regions, 5 ml of blood was collected in gel-labeled tubes. There was no systematic approach to sampling due to the diversity of support and collaboration throughout the study period. Therefore, a different number of samples were collected from random locations in the area.

A. In Human

Blood samples (n=250) were collected randomly during a 6-monthstime of study by venipuncture using a hypodermic needle. Blood samples were collected from females in tubes with EDTA. It was carried out by medical and nursing personnel with individual consent.

B. Storage and Testing

The sample was stored at -20^oC and refrigerated for experimentation in the College of Veterinary Sciences and Animal Husbandry. The blood was centrifuged at 12,000 rpm. Store at -20^oC for 5 minutes. The serum was tested against Brucella antibodies for Brucella spp.

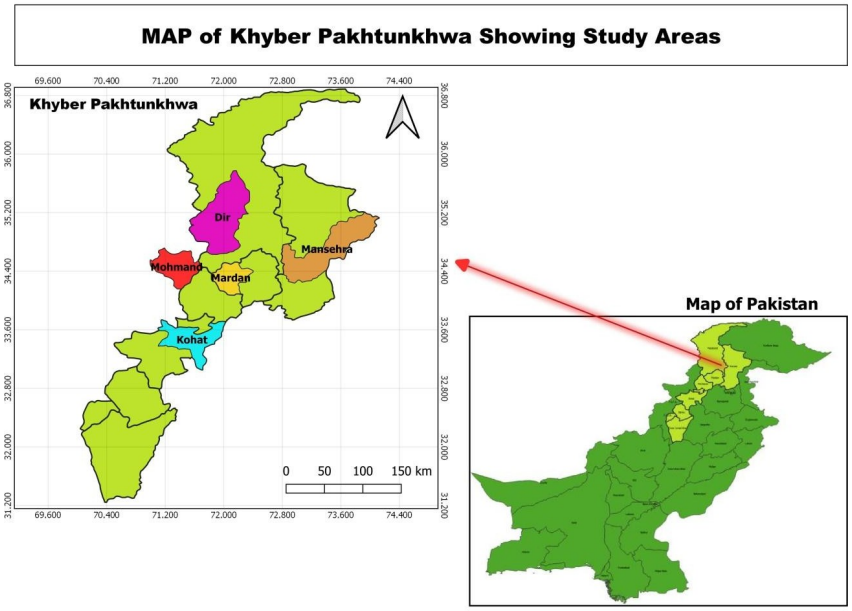

Fig. 1. Map showing the study areas

Fig. 2. The process of collection of blood samples

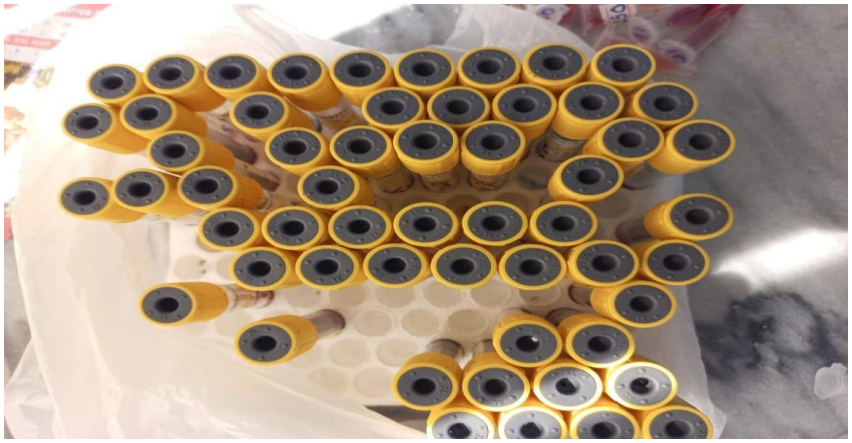

Fig. 3. Samples stored in EDTA tubes

3.4. Materials and Methods

A. Serological Detection of Brucellosis

Brucella Serological Treatment by CELISA (Competitive Enzyme Linked Immunosorbent Assay)

CELISA was performed using a Calibiotech IgG ELISA kit to detect Brucella IgG antibodies in human serum or plasma. To begin the evaluation, all samples were taken at room temperature and mixed gently. The number of the desired strips was place on the tray. A 1:21 dilution of the test sample was made by adding 10μl of the sample to 200μl of diluent. 100μl of diluted serum was calibrated and analyzed in the appropriate wells and then placed in well 1A. After that, at room temperature they were placed in incubator for 20 minutes. Then with 300 micro liter of buffer wash the wells were washed 3 times. Dispense 100μl of Enzyme Conjugate into each well and incubate for 20 min at room temperature. The continuation, Conjugated enzyme was then removed from all wells and then wash them with 300 micro liter of 1X wash buffer for three times. 100μl of TMB substrate was dispensed and then were placed at room temperature for 10 minutes. The further reaction was stopped by adding 100 μl of a quenching solution. Plates were stored in an ELISA plate reader at 450nm for assay. The mean of the positive control group must be greater than 0.250.

Fig. 4. Serum in Epindorf tubes

B. Interpretation

Samples with values less than 0.9 are taken as negative, while samples with values between 0.9 and 1.1 are slightly positive, and values greater than 1.1 are considered positive.

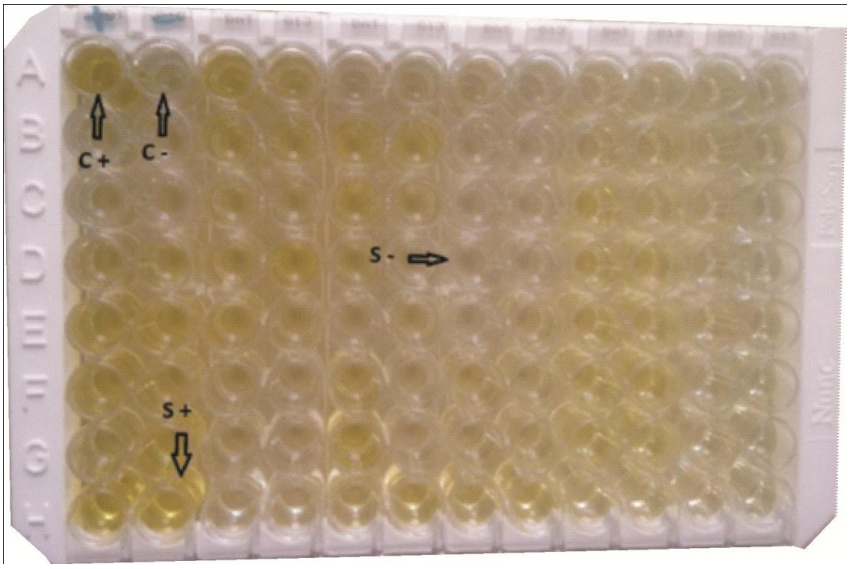

Fig. 5. The ELISA plate showing (Control Positive) C+, (Control Negative) C- and (Sample Positive) S+ and (Sample Negative) S-

4. Data Analysis and Interpretations

The data collected and analyzed during the process are as follows.

Table 1. Prevalence of brucellosis in district mardan

Risk Factors	Categories	Sera Tested	Positive (%)
Geographical Region	Mardan	120	10(8.3%)
Contact with Animals	No	40	0(0%)
Contact with Animals	Yes	80	10(12.5%)
Occupation	Livestock Keepers	80	7(8.75%)
Occupation	Milkers	30	3(10%)
Occupation	Others	10	0(0%)
Gender	Females	120	10(8.3%)
Age	$\leq$30	60	5(8.3%)
Age	$31-50$	40	3(10%)
Age	$\geq$50	20	2(10%)
Consumption of Raw Milk	No	110	0(0%)
Consumption of Raw Milk	Yes	10	10(100%)

4.1. Interpretation

Table 1 shows the data analysis of district Mardan on the basis of different factors. It is evident that out of 120 sera tested 10 samples were positive as the climate of district Mardan is warm and semi-arid and changes in climatic conditions greatly influenced the brucellosis transmission. It is clear from the above table that the people who were within touch with animals were 10(12.5%) positive out of 80. On the basis of occupation the livestock keepers were 7(8.75%) positive, 3(10%) milkers were positive and others who were not in the contact with animals showed no positive records. Age wise prevalence showed that the 5(8.3%) females of the age $\leq$30 were positive, 3(10%) females of the age $31-50$ were positive and 2(10%) females of the $\geq$50 were positive. Those females who were consuming raw milk were all positive.

Table 2 shows the analysis of the data of district Dir on the basis of different factors. Total 15 samples were tested and only 1 was positive because climate of Dir is little cold and changes in climatic conditions greatly influenced brucellosis transmission. It is clear from the above table that those people who were in closed vicinity with animals were 1 (9%) positive out of 11. On the basis of occupation the livestock keepers showed no positive

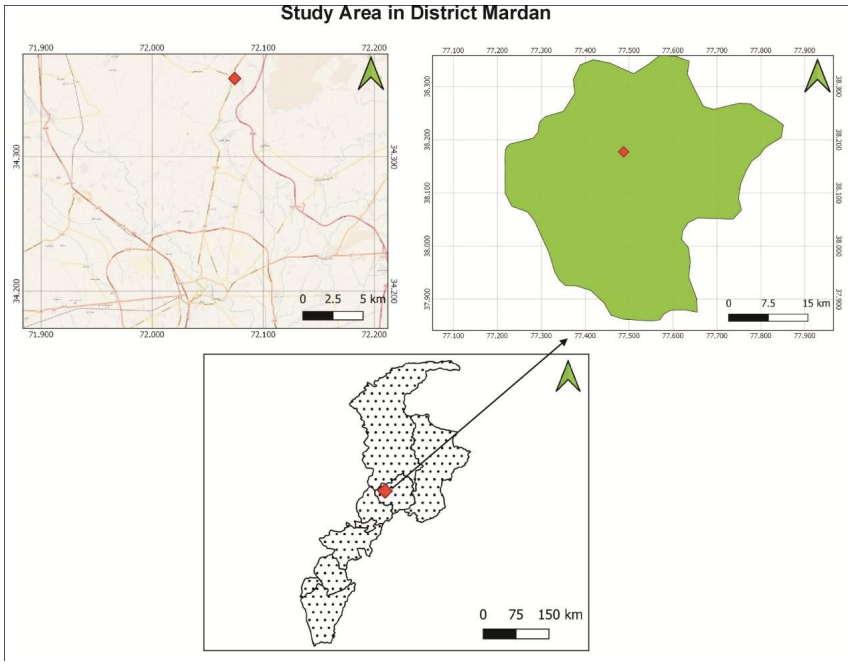

Fig. 6. Study Area in District Mardan

Table 2. Prevalence of brucellosis in district Dir

Risk Factors	Categories	Sera Tested	Positive (%)
Geographical Region	Dir	15	1(6.6%)
Contact with Animals	No	4	0(0%)
Contact with Animals	Yes	11	1(9%)
Occupation	Livestock Keepers	8	0(0%)
Occupation	Milkers	4	1(25%)
Occupation	Others	3	0(0%)
Gender	Females	15	1(6.6%)
Age	≤ 30	11	0(0%)
Age	$31 - 50$	3	1(33.3%)
Age	≥ 50	1	0(0%)
Consumption of Raw Milk	No	14	0(0%)
Consumption of Raw Milk	Yes	1	1(100%)

record while 1 (25%) of milkers were positive and others who were not in the contact with animals showed no positive records. Age wise prevalence showed that the females of the age ≤ 30 were negative while 1(33.3%) female of the age $31 - 50$ was positive and females of the age ≥ 50 were negative. Those females who were consuming raw milk were 100% positive. Hence out of 15 samples only 1 sample was positive.

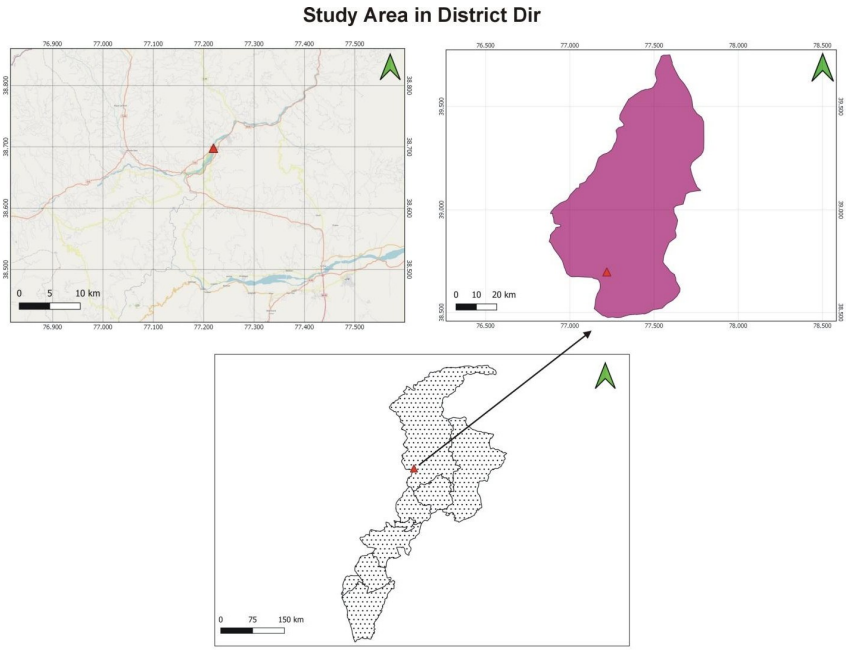

Fig. 7. Study Area in District Dir

Table 3. Prevalence of brucellosis in Mohmand Agency

Risk Factors	Categories	Sera Tested	Positive (%)
Geographical Region	Mohmand Agency	18	2(11.1%)
Contact with Animals	No	5	0(0%)
Contact with Animals	Yes	13	2(15.3%)
Occupation	Livestock Keepers	10	2(20%)
Occupation	Milkers	5	0(0%)
Occupation	Others	3	0(0%)
Gender	Females	18	2(11.1%)
Age	≤ 30	9	1(11.1%)
Age	$31 - 50$	5	0(0%)
Age	≥ 50	4	1(25%)
Consumption of Raw Milk	No	16	0(0%)
Consumption of Raw Milk	Yes	2	2(100%)

Table 3 shows analysis of the data of Mohmand Agency on the basis of different factors. Total 18 samples were tested and only 2 (11.1%) were positive. The climate of Mohmand Agency is cold in winter and hot in summer and the samples were tested in winter and changes in the climate of the regions greatly influenced the brucellosis transmission. It is clear from the above table that the people who were living in close vicinity with different animals were 2 (15.3%) positive out of 13. On the basis of occupation 2 (20%) livestock keepers out of 10 were positive while all milkers were negative and others who were not in the contact with animals were also negative. Age wise prevalence showed that 1 (11.1%) female out of 9 of the age ≤ 30 were positive while females of the age $31 - 50$ were negative and 1 (25%) female of the age ≥ 50 was positive. Those females who were consuming raw milk were 100% positive. Hence out of 18 samples only 2 samples were positive in Mohmand Agency.

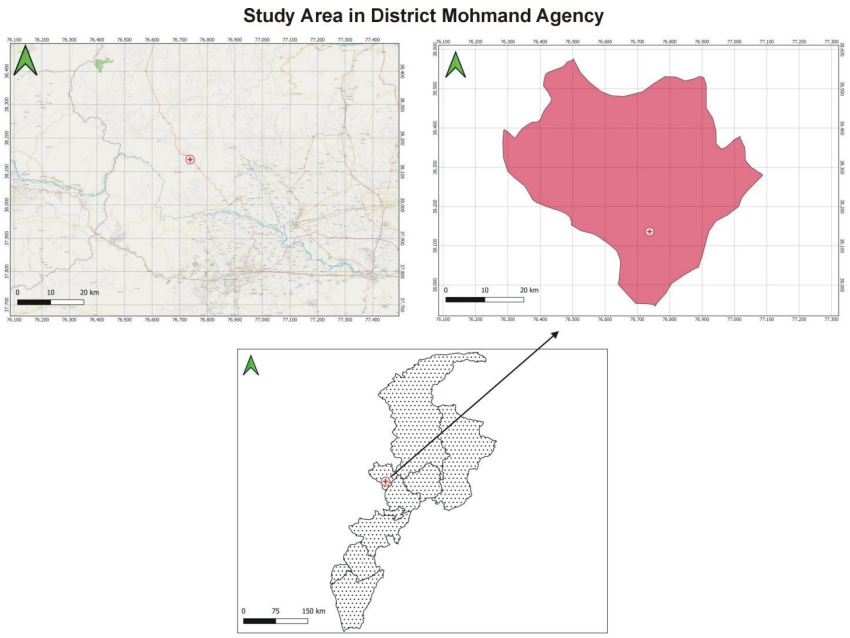

Fig. 8. Study Area in Mohmand Agency

Table 4. Prevalence of brucellosis in District Kohat

Risk Factors	Categories	Sera Tested	Positive (%)
Geographical Region	Kohat	30	3(10%)
Contact with Animals	No	22	1(4.5%)
Contact with Animals	Yes	8	2(25%)
Occupation	Livestock Keepers	15	3(20%)
Occupation	Milkers	12	0(0%)
Occupation	Others	3	0(0%)
Gender	Females	30	3(10%)
Age	≤ 30	21	2(9.5%)
Age	$31 - 50$	5	1(20%)
Age	≥ 50	4	0(0%)
Consumption of Raw Milk	No	27	0(0%)
Consumption of Raw Milk	Yes	3	3(100%)

Table 4 shows data analysis of district Kohat. Out of total 30 sera samples only 3 samples were found positive during summer season in Kohat, as Kohat is warm and semi-arid region and climatic changes greatly influenced brucellosis transmission. Those females who were taking care for animals were more positive 2 (25%). 3 (20%) of livestock keepers were positive while 12 milkers and 3 of others occupation were negative. 2 (9.5%) females of the age $\leq$30 were positive while 1 (20%) female of the age $31-50$ were positive while females of the age $\geq$50 were all negative. 3 out 30 females were consuming raw milk and were (100%) positive. Hence in district Kohat only 3 females out of 30 were positive.

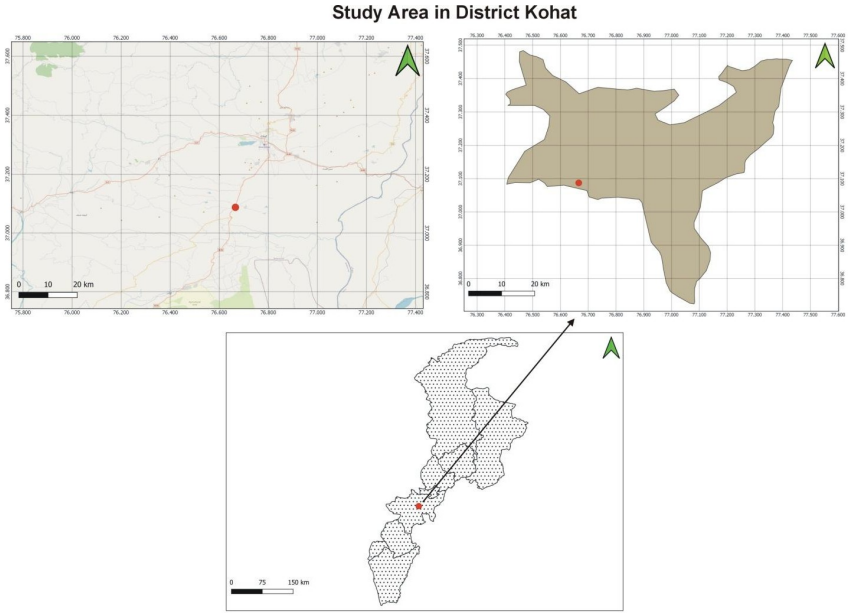

Fig. 9. Study Area in District Kohat

Table 5. Prevalence of brucellosis in District Mansehra

Risk Factors	Categories	Sera Tested	Positive (%)
Geographical Region	Mansehra	17	1(5.8%)
Contact with Animals	No	13	0(0%)
Contact with Animals	Yes	4	1(25%)
Occupation	Livestock Keepers	11	1(9%)
Occupation	Milkers	3	0(0%)
Occupation	Others	3	0(0%)
Gender	Females	17	1(5.8%)
Age	$\leq$30	10	1(10%)
Age	$31-50$	5	0(0%)
Age	$\geq$50	2	0(0%)
Consumption of Raw Milk	No	16	0(0%)
Consumption of Raw Milk	Yes	1	1(100%)

Table 5 shows the data analysis of district Mansehra. 17 samples were collected from district Mansehra in winter and only 1 (5.8%) sample was found positive as climate of the region is cold and changes in climatic conditions greatly influenced transmission of brucellosis. 1 (25%) female out of 4 were positive as they were in close contact with animals. Among 11 livestock keepers 1 (9%) was positive while milkers and females of other occupations were all negative. Only 1 (10%) female out of 10 of the age $\leq$30 was positive while females of the age 31-50 and $\geq$50 were all negative. Only 1 (100%) female was consuming raw milk and was found positive.

The above table shows overall prevalence of brucellosis in five districts of KP Pakistan. A number of 200samples were collected randomly from five districts of KP Pakistan. The overall prevalence was found 8.5% by ELISA.

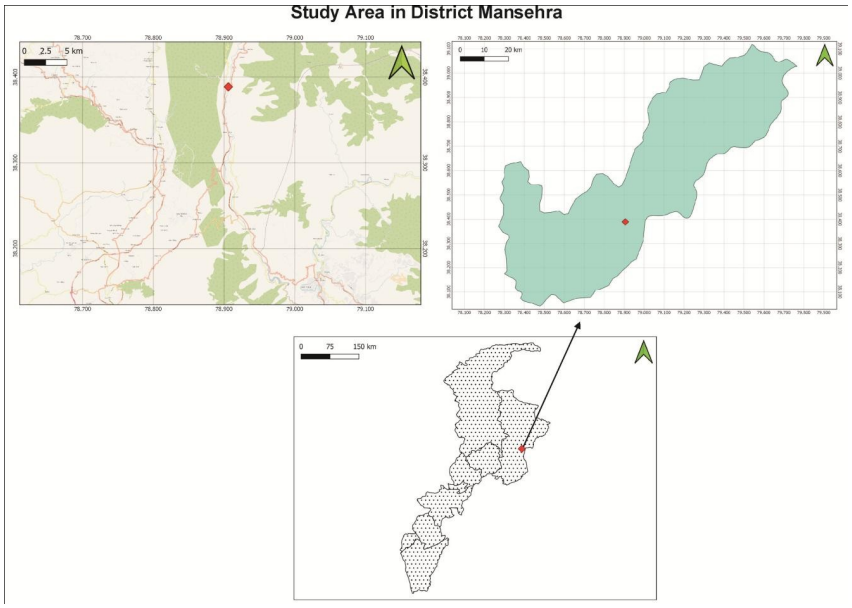

Fig. 10. Study Area in District Mansehra

Table 6. Overall prevalence of brucellosis in five districts of Khyber Pakhtunkhwa, Pakistan

Risk Factors	Categories	Sera Tested	Positive (%)	P-value
Geographical Region	Mardan	120	17(8.5%)	0.976
Geographical Region	Dir	30	17(8.5%)	0.976
Geographical Region	Mohmand Agency	15	17(8.5%)	0.976
Geographical Region	Kohat	17	17(8.5%)	0.976
Geographical Region	Mansehra	18	17(8.5%)	0.976
Contact with Animals	No	84	1(1.19%)	0.001
Contact with Animals	Yes	116	16(13.7%)	0.001
Occupation	Livestock Keepers	124	12(9.6%)	0.315
Occupation	Milkers	54	5(9.2%)	
Occupation	Others	22	0(0%)	
Age	≤ 30	111	9(8.1%)	0.961
Age	$31 - 50$	58	5(8.6%)	0.961
Age	≥ 50	31	3(9.6%)	0.961
Consumption of Raw Milk	Yes	17	17(100%)	0.00001
Consumption of Raw Milk	No	183	0(0%)	0.00001

Prevalence of brucellosis was high i.e. 13.7% in females who were in close contact with animals as compare to those who were not in close contact with animals and the prevalence in them is 1.19%.

Prevalence on the basis of occupation was more in livestock keepers which was 13.7% whereas prevalence recorded in milkers was 9.2% and in females of other occupation the prevalence was 0%.

Age wise prevalence of brucellosis in females was recorded 8.1%, 8.6% and 9.6% in age groups $\leq$30, $31-50$ and $\geq$50.

Prevalence was recorded in females who were consuming raw milk. Total 17 females were consuming raw milk and were all positive while those who were consuming pasteurized milk were all negative.

5. Discussion

Brucellosis is the most common animal disease in the world (Papas, Papadimitriou et al., 2006), and is caused by bacteria (Godfroid, Scholz et al., 2011) and are responsible for the pathogenesis of brucellosis and have the ability to survive and multiply in the cells of the phagocytic system (Jarvis et al., 2002). Brucellosis is the most advanced and undiagnosed infection worldwide, as well as in Pakistan (Rubach et al., 2013; WHO, 2012). A variety of mammals, including humans, cattle, sheep, goats, camels, pigs, and wildlife are targets of brucellosis (Cutler et al. 2005). The diagnostic tool used for the serological detection of brucellosis in the current study is ELISA. The aim of the conducted study is to determine the prevalence of the brucellosis in females (human) of different districts of KP Pakistan. Total prevalence in this study is 8.5%. The prevalence of brucellosis in females (human), 9.33% by SPAT was recorded by Din et al., 2013. Nusrat et al (2004) also recorded the same findings. Nikokar et al (2011), Otlu et al., (2008) calculated prevalence of brucellosis and reported it as 3.2%. Mohmand et al (2012) recorded prevalence of 6.09% in female population of Pakistan involved in animal keeping. Parveen and Raqeebullah (2015) calculated 8% of prevalence in females of KP Pakistan. In the present study the value of prevalence recorded is high as compare to other studies in different districts of KP Pakistan. It is because of the reason that in the present study only those females were targeted who were involved in farming with their males, milking, keeping animals, processing of dairy products and different strategies of animal feeding. Furthermore difference in the studies observed may also be due to the great differences in the environmental conditions and climate of the studied regions and we studied earlier that the changes in the climatic conditions of the different regions greatly influenced the brucellosis transmission. Area wise prevalence recorded was 8.5% in the different districts of KP Pakistan. Out of total 200 samples 120 samples were collected from district Mardan. Among 120 samples only 10 samples were positive in tehsil Katlang because of the greater involvement of females in animal keeping and due to hot climate as season greatly affect transmission of brucellosis. 30 samples were collected from the females of Kohat who were involved in animal keeping, milking, management of dairy products and working in different fields with their males. 3 females who were found to be positive were involved in all these activities while those who were not in touch with animals were negative. 15 samples were collected from Dir and only 1 was positive and remaining 14 sera samples were negative because of the cold climate and only limited females were involved in farming and keeping of animals. 17 samples were collected from Mansehra and only 1 was positive and same in Mohmand Agency only 2 samples were positive out of 18. It was observed that more positive samples were found in district Mardan and tehsil Katlang which is rural area and females are mostly involved in animals husbandry. Out of 200, 116 females were in contact with animals and 16 females were positive as they were unaware of the knowledge of managing animals in proper way while all other females have the knowledge of handling animals in proper way and were protected from the infection. Females under the age of 30 were more positive as compared to other age groups. Most of these females were in close contact with animals and were working in fields with their males and utilizing animals dairy products. Hence there is a need to educate them and aware them of handling animals in proper way. The conclusions and results of our study are in similarity with the study conducted in Pakistan (Mukhtar 2008). However variations observed in the age groups was not statistically significant. Changes in the values of prevalence of brucellosis on the basis of different occupations has been discussed and showed in table 6. Livestock keepers were mostly identified as sero positive (9.6%). Out of 124 livestock keepers only 12 were positive; in 54 milkers only 5 were positive. Similar findings were reported where farmers and animal handlers were at high risk (Abo Shehada et al., 1996).

6. Conclusion and Recommendations

Studies have shown that the prevalence of this condition is higher in women than in men. Therefore, it can be concluded that brucellosis remains a major health issue in villages and well-implemented strategies are needed to control and eradicate the disease in these areas. It is recommended to limit and improve the slaughter and

movement of infected animals. Hygiene and organization of the herd to avoid mixing and types of animals. Implementation of biosafety practices together with mass vaccination. One or more tests are required. RBPT to diagnose the disease and ELISA and PCR as a confirmatory test.

7. Conflict of Interest

The authors declare that there are no conflict of interests, we do not have any possible conflicts of interest.

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